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(54) Title: PHARMACEUTICAL COMPOSITION FOR COMBINATION THERAPY OF HORMONE DEPENDENT CANCERS

(57) Abstract

A method of treating sex steroid dependent cancers in a warm-blooded animal which comprises administering a pharmaceutically effective amount of an antiandrogen and/or an antiestrogen and/or at least one inhibitor of sex steroid biosynthesis to the animal whose hormone output of the testes or ovaries, respectively, is blocked, with the proviso that the method of treating prostate cancer comprises administering pharmaceutically effective amounts of an antiandrogen and at least one inhibitor of sex steroid biosynthesis and with the further proviso that, if antiestrogen is used in treating breast cancer it is used in association with an antiandrogen and/or at least one inhibitor of sex steroid biosynthesis. The invention further relates to kits containing pharmaceutical compositions containing the active ingredients to be used in the novel combination therapy.

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PHARMACEUTICAL COMPOSITION FOR COMBINATION THERAPY OF HORMONE DEPENDENT CANCERS

This invention relates to a combination therapy of sex steroid dependent cancers in susceptible warm-blooded animals including humans comprising administering to such animals whose hormone output of their testes or ovaries respectively is blocked a pharmaceutically effective amount of an antiandrogen and/or an antiestrogen and/or at least one inhibitor of sex steroid biosynthesis, with the proviso that the method of treating prostate cancer comprises administering pharmaceutically effective amounts of an antiandrogen and at least one inhibitor of sex steroid biosynthesis and the method of treating breast cancer comprises administering a pharmaceutically effective amount of an antiandrogen or of at least one inhibitor of sex steroid biosynthesis or of an antiandrogen and an antiestrogen or of an antiandrogen and at least one inhibitor of sex steroid biosynthesis or of an antiestrogen and at least one inhibitor of sex steroid biosynthesis or of an antiandrogen and an antiestrogen and at least one inhibitor of sex steroid biosynthesis. In the therapy of sex steroid dependent cancers such as testicular cancer, ovarian cancer, colon-r ctal cancer, renal cancer, pancreatic cancer,

liver cancer, stomach cancer, skin cancer, ut rine cancer, brain cancer and larynx cancer all above listed combinations are applicable. The hormone output of the animal's testes or ovaries, respectively, can be blocked by surgical or chemical means. (In post-menopausal women the attending clinician may decide that the hormone output of the ovaries need not be blocked.)

While various investigators have been studying hormone-dependent breast and prostate cancer, none have proposed the combination therapy of this invention.

A. V. Schally et al., Cancer Treatment Reports, 68, (No. 1) 281-289 (1984), summarize the results of animal and clinical studies on growth inhibition of hormone-dependent mammary and prostate tumors by use of analogues of luteinizing hormone-releasing hormones, the so-called LH-RH agonists and suggest that LH-RH analogs and/or antagonists may have potential for treating breast cancer.

T. W. Redding and A. V. Schally, Pro. Natl. Acad. Sci. USA, 80, 1459-1462 (1983), disclose reduction of estrogen-dependent mammary tumors in rats and mice by use of an LH-RH agonist, [D-Trp^6]LH-RH or of two specific antagonists and inhibition of prostate tumor growth in rats of chronic use of an LH-RH agonist, [D-Trp^6] LH-RH.

In U.S. Patent 4,071,622, it is disclosed that use of certain LH-RH agonists causes regression of DMBA-induced mammary carcinoma in rats.

In U.S. Patent 4,472,382, it is disclosed that prostate adenocarcinoma, benign prostate hypertrophy and hormone-dependent mammary tumors may be tr ated with various LH-RH agonists and that prostate

ad nocarcinoma and benign hypertrophy may b treated by use of various LH-RH agonists and an antiandrogen. However, there is no suggestion or disclosure of the present invention.

Some clinical improvement in premenopausal women with breast cancer by use of the two LH-RH agonists, Buserelin and Leuprolide, is also reported by H.A. Harvey et al. "LH-RH analogs in the treatment of human breast cancer", LH-RH and its Analogs - A New Class of Contraceptive and Therapeutic Agents (B.H. Vickery and J.J. Nestor, Jr., and E.S.E. Hafez, eds) Lancester, MTP Press, (1984) and by J.G.M. Klijn et al. "Treatment with luteinizing hormone releasing hormone analogue (Buserelin) in premenopausal patients with metastatic breast cancer", Lancet, 1, 1213-1216 (1982).

Treatment of advanced breast cancer with aminoglutethimide after therapy with the antiestrogen, Tamoxifen is disclosed by A.V. Buzdar et al., Cancer, 50, 1708-1712 (1982).

H. Flax et al., Lancet, 1204-1207, (1973), suggest some women's breast cancers are androgen-dependent.

In U.S. Patent 4,329,364, it is disclosed that the antiandrogen, 4'-nitro-3'-trifourormethyl isobutyranilide may be used for treatment of prostatic cancer.

Some clinical improvement in men with prostate cancer by use of the two LH-RH agonists, Buserelin and Leuprolide, is also reported by N. Faure et al. at pages 337-350 and by R.J. Santen et al. at pages 351-364, respectively, LH-RH and its Analogs - A New Class of Contraceptive and Therapeutic Agents (B.H. Vickery and J.J. Nestor, Jr., and E.S.E. Hafez, eds) Lancester, MTP Press, (1984).

R. Santen t al., The Journal of Steroid Biochemistry, Volume 20, No 6B, at page 1375 (1984), disclose that use of ketoconazole in combination with chronic administration of Leuprolide in rodents decreased basal and Leuprolide stimulated testosterone levels.

D. Kerle et al., The Journal of Steroid Biochemistry, Volume 20, No 6B, at page 1395 (1984) disclose that the combined use of a LH-RH analogue and ketoconazole produced objective responses in some prostate cancer patients who have relapsed or failed to respond to treatment with a LH-RH analogue alone.

F. Labrie et al., Abstracts of 7th International Congress of Endrocrinology, Excerpta Medica (1984) at page 98 discloses that treatment of prostate cancer patients with LH-RH agonists alone causes a transient increase in serum androgen levels lasting for 5 to 15 days before castration levels are reached. While F. Labrie et al. recommends that orchiectomy, estrogen and LH-RH agonists alone should not be further used for treatment of prostate cancer in the absence of a pure antiandrogen, there still is a need for a method of treatment of prostate cancer that effects more complete androgen blockage at the start as well as during the full period of treatment.

F. Labrie et al., The Prostate, 4, 579-594 (1983), disclose that use of a combination therapy of an LH-RH agonist (Buserelin) and an antiandrogen (Anandron) to treat advanced prostate cancer in previously untreated patients effects simultaneous inhibition of androgens of both testicular and adrenal origin.

F. Labrie et al., J. Steroid Biochem., 19, 99-1007 (1983), disclose the treatment of prostat

cancer by the combined administration of an LH-RH agonist and an antiandrogen. Labrie et al. disclose animal and clinical data in support of the proposition that the combined LH-RH/antiandrogen treatment neutralizes the stimulatory influence of all androgens on the development and growth of androgen-dependent prostatic cancer.

In U.S. Patent 4,094,994, it is disclosed that the use of antiestrogens such as meso-3,4-bis(3'-hydroxyphenyl)hexane inhibits MCF7 human breast tumor cells. In fact, the inhibitory activity of the anti-estrogen was antagonized by estradiol.

H. Mouridsen et al,. Cancer Treatment Review 5, 131-141, (1978), disclose that Tamoxifen, an antiestrogen is effective in remission of advanced breast cancer in about 30% of the patients treated.

J.G.M. Klijn et al., (J. Steroid Biochem, Vol. 20 (No. 6B), 1381 (1984), disclosed the combined use of the antiestrogen, Tamoxifen, and the LH-RH agonist, Buserelin, for treatment of breast cancer is known, but objective remission of such cancers remains low (35%).

The present invention provides a new combination therapy of sex steroid dependent cancers which inhibits tumor growth and metastases and causes in some instances complete remission. The invention is exemplified by detailed discussion of the therapy of breast cancer and prostate cancer. The therapy of other sex steroid dependent cancers (listed above) is carried out analogously.

In one aspect, the invention provides a method of treating sex steroid dependent cancers such as, for example, breast cancer in a warm-blooded female animal in need of such treatment which comprises

administering to said animal whose hormon output of the ovaries is blocked, a therapeutically effective amount of an antiandrogen or a pharmaceutical composition thereof. The ovarian hormonal secretions of said animal can be blocked by surgical or chemical means. In one aspect, the invention provides a method of treating sex steroid dependent cancers such as, for example, breast cancer in a castrated warm-blooded female animal, i.e., such a female animal whose ovaries are blocked by surgical or chemical means from secreting estrogen, which comprises administering to such a female in need of such treatment an antiandrogen in association with at least one inhibitor of sex steroid biosynthesis and, optionally, an antiestrogen, or pharmaceutical compositions thereof, in amounts sufficient to treat such cancers, e.g., breast cancer. In another aspect, the invention provides a method of treating sex steroid dependent cancers such as, for example, breast cancer in a warm-blooded female animal in need of such treatment which comprises blocking the ovarian hormonal secretions of said animal by surgical or chemical means and administering to said animal therapeutically effective amounts of an antiestrogen in association with at least one inhibitor of sex steroid biosynthesis, or pharmaceutical compositions thereof. In one aspect, the invention provides a method of treating sex steroid dependent cancers such as, for example, breast cancer in a warm-blooded female animal whose ovaries are blocked by surgical or chemical means from secreting estrogen, which comprises administering to an animal in need of such treatment an antiandrogen and an antiestrogen, or pharmaceutical compositions thereof, in amounts sufficient to treat such cancers, e.g., breast cancer.

By complet ly blocking sex steroids (androgens and estrogens) production and/or action, the present invention provides a method of inhibiting the growth of sex steroid-sensitive tumors, e.g., breast tumors in warm-blooded animals having such tumors.

In female mammals, the ovaries may be surgically removed (oophorectomy) but preferably the secretion of estrogens from the ovaries is blocked by chemical castration by administering an effective amount of an LH-RH agonist or antagonist. Thus, in a preferred aspect, the present invention provides a method of treating sex steroid dependent cancers such as, for example, breast cancer in a warm-blooded female animal, which comprises administering to such a female in need of such treatment an LH-RH agonist or antagonist, in association with an antiandrogen and/or an antiestrogen and/or at least one inhibitor of sex steorid biosynthesis according to the invention, preferably an antiandrogen. Preferably in this treatment at least one inhibitor of sex steroid biosynthesis, or pharmaceutical compositions thereof, and/or antiestrogen is administered.

In its preferred aspect, the LH-RH agonist is administered parenterally (subcutaneously or intramuscularly or intranasally) and, in association therewith, the antiandrogen and the inhibitor of sex steroid biosynthesis and/or antiestrogen are each administered orally.

Thus, this invention provides a novel method for effective treatment of sex steroid dependent cancers such as, for example, female breast cancer, in the absence of antiestrogen. In addition, the amounts of antiestrogen required when administered in association with this combined therapy are low r than

normally used in prior art methods, e.g., J.G.M. Klijn et al., J. Steroid Biochem. 20 (No. 6B) 1381 (1984), to treat breast cancer, and thus, the harmful effects of relatively large doses of antiestrogen are minimized.

In one preferred aspect, the present invention provides an effective method of treating sex steroid dependent cancers such as, for example, breast cancer in warm-blooded female animals in need of such treatment by administering an LH-RH agonist or antagonist, in association with an antiandrogen and an inhibitor of sex steroid biosynthesis or pharmaceutical compositions thereof in amounts sufficient to inhibit tumor growth. These active compounds can be administered together or in any order as discussed hereinafter.

To assist in determining the effect of the treatment, blood plasma concentrations of the sex steroids of adrenal and ovarian origin, i.e., precursor steroids, androgens and estrogens, and tumor size are measured. Lowered concentrations of sex steroids and reduction in tumor size are indicative of successful treatment, e.g. inhibition of tumor growth using active compounds described herein in accordance with the present invention. The concentrations of adrenal androgens and estrogens such as dehydroepiandrosterone (DHEA), DHEA-sulfate (DHEAS), androst-5-ene-3β,17β-diol (Δ^5-diol) and, the ovarian estrogen, 17β-estradiol (E_2) are measured by standard methods well known to those skilled in the art, see for example F. Labrie et al., The Prostate, 4, 579-594 (1983).

The change in tumor size is measured by standard physical methods well known to those skilled

in the art, .g., bone scan, chest X-ray, skeletal survey, ultrasonography of the liver and liver scan (if needed), CAT-scan and physical examination.

While a LH-RH agonist or a LH-RH antagonist may be used in one preferred aspect of the present invention, the use of a LH-RH agonist is more preferred.

In another aspect, the invention provides a method of treating sex steroid dependent cancers (excluding prostate cancer) such as, for example, breast cancer in a warm-blooded male animal in need of such treatment which comprises blocking the testicular hormonal secretions of said animal by surgical or chemical means and administering to said animal a therapeutically effective amount of an antiandrogen, or a pharmaceutical composition thereof. In another aspect, the invention provides a method of treating sex steroid dependent cancers (excluding prostate cancer) such as, for example, breast cancer in a castrated warm-blooded male animal, i.e., such a male animal whose testes are blocked by surgical or chemical means from secreting androgen, which comprises administering to an animal in need of such treatment therapeutically effective amounts of an antiandrogen in assocciation with at least one inhibitor of sex steroid biosynthesis, or a pharmaceutical composition thereof, in an amount sufficient to treat the cancer. In another aspect, the invention provides a method of treating sex steroid dependent cancers (excluding prostate cancer) such as, for example, breast cancer in a castrated warm-blooded male animal, which comprises administering to an animal in need of such treatment therapeutically effective amounts of an antiandrogen and an antiestrogen, optionally in association with at

least on inhibitor of s x st roid biosynth sis. By simultaneously blocking sex-steroids (androgens and estrogens of testicular and adrenal origin) production and/or action, the present invention provides a method of inhibiting the growth of sex steroid-sensitive (breast) tumors in warm-blooded male animals having such tumors.

In male mammals, the removal of testicular androgens may be achieved by surgical castration but preferably the secretion of androgens from the testes is blocked by chemical castration by administering to the warm-blooded male animal, an effective amount of an LH-RH agonist or antagonist. Thus, in a preferred aspect, the present invention provides a method of treating sex steroid dependent cancers (excluding prostate cancer) such as, for example, breast cancer in a warm-blooded male animal, which comprises administering to an animal in need of such treatment an LH-RH agonist or antagonist in association with an antiandrogen and/or an antiestrogen and/or at least one inhibitor of sex steroid biosynthesis as discussed in the preceding paragraph, preferably in association with an antiandrogen, or a pharmaceutical composition thereof, in amounts sufficient to treat the cancer.

In its preferred aspect, the LH-RH agonist or antagonist is administered parenterally (subcutaneously or intramuscularly) and the antiandrogen, the antiestrogen and the inhibitor(s) of sex steroid biosynthesis (if used) are administered orally.

In one preferred aspect, the present invention provides an effective method of treating sex steroid dependent cancers (excluding prostate cancer) such as, for example, breast cancer in warm-blooded male animals in need of such treatment by administering

a LH-RH agonist or antagonist in association with an antiandrogen or pharmaceutical compositions thereof in amounts sufficient to inhibit tumor growth. The use of an LH-RH agonist in association with an antiandrogen produced almost complete blockage of testicular steroid secretion while simultaneously blocking about 25 to 60% of the precursor sex steroids (androgens and estrogens) of adrenal origin. See Figure 3 and Example 1. In still another preferred aspect, at least one inhibitor of sex steroid biosynthesis is administered to warm-blooded male animals having sex steroid dependent cancers (excluding prostate cancer) such as, for example, breast cancer in association with surgical castration or chemical castration (by use of an LH-RH agonist or antagonist) and the antiandrogen. These active compounds can be administered together or in any order as discussed hereinafter.

To assist in determining the effect of the treatment, blood plasma concentrations of the testical and adrenal androgens and estrogens and tumor size are measured. Lowered concentrations of sex steroids and precursors and reduction in tumor size are indicative of successful treatment, e.g. inhibition of tumor growth. The concentrations of adrenal androgens and estrogens such as dehydroepiandrosterone (DHEA), DHEA-sulfate (DHEAS), androst-5-ene-3β,17β-diol (Δ^5-diol) and, the estrogen, 17β-estradiol (E_2) are measured by standard methods well known to those skilled in the art, see for example F. Labrie et al., The Prostate, 4, 579-594 (1983).

The active compounds described herein and used in accordance with the present invention exhibited tumor growth inhibition and lowered concentrations of sex steroids when tested in warm-blooded male animals

including man. Se Figure 1-2 and 3 and th descriptions thereof in Example 1.

The change in tumor size is measured by standard physical methods well known to those skilled in the art, e.g., bone scan, chest X-ray, skeletal survey, ultrasonography of the liver and liver scan (if needed), CAT-scan and physical examination.

While a LH-RH agonist or a LH-RH antagonist may be used in one preferred aspect of the present invention, the use of a LH-RH agonist is more preferred.

In another aspect, the invention provides a method of treating sex steroid dependent cancers such as, for example, prostate cancer in a warm-blooded male animal including humans in need of such treatment which comprises blocking the testicular hormonal secretions of said animal by surgical or chemical means and administering to said animal a therapeutically effective amount of an antiandrogen in association with at least one inhibitor of sex steroid biosynthesis, or pharmaceutical compositions thereof. By simultaneously blocking androgens of testicular and adrenal origin production and/or action, the present invention provides a method of inhibiting the growth of sex steroid-sensitive carcinomas in warm-blooded male animals having such carcinomas.

In male mammals, the removal of testicular androgens may be achieved by surgical castration (orchiectomy) but preferably the secretion of androgens from the testes is blocked by chemical castration by administering to warm-blooded male animal, an effective amount of an LH-RH agonist or antagonist. In a preferred aspect, the present invention provides a method of tr ating s x steroid dependent cancers such

as, for example, prostate cancer in a warm-blooded male animal having such cancer, which comprises administering to said animal therapeutically effective amounts of an LH-RH agonist or antagonist in association with an antiandrogen and at least one inhibitor of sex steroid biosynthesis, or pharmaceutical compositions thereof.

In its preferred aspect, the LH-RH agonist is administered parenterally (subcutaneously or intramuscularly) and the antiandrogen and the inhibitor(s) or inhibitors of sex steroid biosynthesis are each administered orally.

In one preferred aspect, the present invention provides an effective method of treating sex steroid dependent cancers such as, for example, prostate cancer in warm-blooded male animals in need of such treatment by administering a LH-RH agonist or antagonist in association with an antiandrogen and an inhibitor of sex steroid biosynthesis of testicular origin or pharmaceutical compositions thereof in amounts sufficient to inhibit such cancer. By use of an inhibitor of testicular sex steroid biosynthesis in association with an antiandrogen and a LH-RH agonist or antagonist more complete androgen blockage is effected at an early stage in the treatment of such (particularly prostate) cancer than achieved by prior art methods. If an inhibitor of sex steroid biosynthesis of both testicular and adrenal origin (such as, for example, aminoglutethimide) is used, the administration of the inhibitor is started on the first day of treatment and continued thereafter to block adrenal steroid synthesis.

In another preferred aspect, the use of an LH-RH agonist or antagonist in association with an

antiandrog n and two inhibitors of sex steroid biosynthesis, e.g. an inhibitor of adrenal sex steroid biosynthesis such as aminoglutethimide and an inhibitor of testicular sex steroid biosynthesis such as ketoconazole, produced almost complete blockage of testicular steroid secretion while simultaneously blocking the precursor sex steroids (androgens and estrogens) of adrenal origin at an early stage of the treatment. While such complete blockage of androgen secretion and/or action has not been achieved by prior art methods, such complete blockage can be achieved and maintained throughout the period of treatment in accordance with the present invention. These active compounds can be administered together or in any order as discussed hereinafter.

To assist in determining the effect of the treatment, blood plasma concentrations of the adrenal and testical androgens and estrogens and parameters of cancer evolution are measured according to known methods. Lowered concentrations of sex steroids and reduction in (prostate or metastatic) tumor size are indicative of successful treatment, e.g. inhibition of prostatic cancer cell growth. The concentrations of adrenal steroids such as dehydroepiandrosterone (DHEA), DHEA-sulfate (DHEAS), androst-5-ene-3β,17β-diol (Δ^5-diol) and, the estrogen, 17β-estradiol (E_2) are measured by standard methods well known to those skilled in the art, see for example F. Labrie et al., The Prostate, 4, 579-594 (1983).

Prostatic size is measured by rectal examination and by transrectal ultrasonography. Objective assessment of the effect of the treatment is also measured by standard physical methods well known to those skill d in the art, e.g., bone scanning, X-

ray, sk letal surv y, intravenous pyelography, CAT-scan and physical examination. The response criteria for prostate cancer developed by the U.S.A. National Prostate Cancer Project (The Prostate, 1, 375-382) may also be used.

The use of therapeutically effective amounts of the inhibitor of testicular sex steroid biosynthesis such as ketoconazole or aminoglutethimide in association with the antiandrogen and the LH-RH agonist or LH-RH antagonist in accordance with the present invention effectively surpresses the serum sex steroid levels, especially serum testosterone and dihydrotestosterone concentrations stimulated by treatment with LH-RH agonist at the start of the treatment even in the presence of an antiandrogen.

While a LH-RH agonist or a LH-RH antagonist may be used in one preferred aspect of the present invention, the use of a LH-RH agonist is more preferred.

The components used in the above described aspects of the invention are described in more detail:

By the term "LH-RH agonist" is meant synthetic analogues of the natural luteinizing hormone-releasing hormone (LH-RH), a decapeptide of the structure:

L-pyroglutamyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-glycyl-L-leucyl-L-arginyl-L-prolylglycyl-NH_2

Typical suitable LH-RH agonists include nonapeptides and decapeptides represented by the formula:

L-pyroglutamyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-X-Y-L-arginyl-L-prolyl-Z

wherein X is D-tryptophyl, D-leucyl, D-alanyl, iminobenzyl-D-histidyl, 3-(2-naphthyl)-D-alanyl, O-tert-butyl-D-seryl, D-tyrosyl, D-lysyl, D-phenylalanyl or N-methyl-D-alanyl and Y is L-leucyl, D-leucyl, N^{α}-methyl D-leucyl, N^{α}-methyl-L-leucyl or D-alanyl and wherein Z is glycyl-NHR_1 or NHR_1 wherein R_1 is H, lower alkyl or lower haloalkyl. Lower alkyl includes straight or branched chain alkyls having 1 to 6 carbon atoms, e.g., methyl, ethyl, propyl, pentyl or hexyls, iso-butyl, neopentyl and the like. Lower haloalkyl includes straight and branched chain alkyls of 1 to 6 carbon atoms having a halogen substituent, e.g., $-CF_3$,-CH_2CF_3, $-CF_2CH_3$. Halogen means F, Cl, Br, with F being preferred.

Preferred nonapeptides wherein Y is L-leucyl and X is an optically active D-form of selected amino acids and Z is NHC_2H_5 are [D-Trp^6, des-gly-$NH_2{}^{10}$]-LH-RH ethylamide (X = D-Trp^6); [D-Ser-(t-BuO)^6, des-gly-$NH_2{}^{10}$]- LH-RH ethylamide [X = D-Ser(t-BuO^6)]; [D-Leu^6, des-gly-$NH_2{}^{10}$]-LH-RH ethylamide (X = D-Leu^6), [D-His(Bzl)^6, des-gly-$NH_2{}^{10}$]LH-RH ethylamide (X = iminobenzyl-D-His^6) and [D-Ala^6, des-gly-$NH_2{}^{10}$]-LH-RH ethylamide (X = D-Ala^6).

Preferred decapeptides include [D-Trp^6]-LH-RH wherein X = D-Trp, Y = L-leucyl, Z = glycyl-NH_2, [D-Phe^6]-LH-RH wherein X = D-phenylalanyl, Y = L-leucyl and Z = glycyl-HN_2) or [D-Nal(2)^6]LH-RH which is [(3-(2-naphthyl)-D-Ala^6]LH-RH wherein X = 3-(2-naphthyl)-D-alanyl, Y = L-leucyl and Z = glycyl-NH_2.

Other LH-RH agonists useful within the scope of this invention are the α-aza analogues of the natural LH-RH, especially, [D-Phe^6,$Azgly^{10}$]-LH-RH, [D-Tyr(M)^6, $Azgly^{10}$]-LH-RH, and [D-Ser-(t-BuO)^6,

Azgly^{10}]-LH-RH disclos d by A.S. Dutta t al. in J. Med. Chem., 21, 1018 (1978) and US Patent 4,100,274 as well as those disclosed in U.S. Patent Nos. 4,024,248 and 4,118,483.

Typical suitable LH-RH antagonists include [N-Ac-D-p-Cl-Phe^{1,2}, D-Phe^3, D-Arg^6, D-Ala^{10}]-LH-RH disclosed by J. Ercheggi et al., Biochem. Biophys. Res. Commun. 100, 915-920, (1981); [N-Ac-D-p-Cl-Phe^{1,2}, D-Trp^3, D-Arg^6, D-Ala^{10}] LH-RH disclosed by D.H. Coy et al., Endocrinology, 110: 1445-1447, (1982); [N-Ac-D-(3-(2-naphthyl)-Ala)^1, D-p-Cl-Phe^2, D-Trp^3, D-hArg(Et$_2$)^6,D-Ala^{10}]-LH-RH and [N-Ac-Pro^1, D-p-F-Phe^2, D-(3-(2-naphthyl)Ala^{3,6}]-LH-RH disclosed by J.J. Nestor et al. J. Steroid Biochem., 20 (No. 6B), 1366 (1984); the nona - and decapeptides analogs of LH-RH useful as LH-RH antagonists disclosed in U.S. Patent 4,481,190 (J.J. Nestor et al.); analogs of the highly constrained cyclic antagonist, cycle [Δ^3 Pro^1, D-p-Cl-Phe^2, D-Trp^{3,6}, N-Me-Leu^7, β-Ala^{10}]-LH-RH disclosed by J. Rivier, J. Steroid Biochem., 20, (No. 6B), 1365 (1984), and [N-Ac-D-(3-(2-naphthyl)-Ala^1, D-p-F-Phe^2, D-Trp^3, D-Arg^6]-LH-RH disclosed by A. Corbin et al., J. Steroid Biochem. 20 (No. 6B) 1369 (1984).

Other LH-RH agonist and antagonist analogs are disclosed in LH-RH and Its Analogs, B.H. Vickory et al. editors at pages 3-10 (J.J. Nestor), 11-22 (J. Rivier et al.) and 23-33 (J.J. Nestor et al.)

The LH-RH agonists and antagonists useful in this invention may conveniently be prepared by the method described by Stewart et al. in "Solid Phase Peptide Synthesis" (published in 1969 by Freeman & Co., San Francisco, page 1) but solution phase synthesis may also be used.

The nona- and decapeptides used in this invention are conveniently assembled on a solid resin support, such as 1% cross-linked Pro-Merrifield resin by use of an automatic peptide synthesizer. Typically, side-chain protecting groups, well known to those in the peptide arts, are used during the dicyclohexylcarbodiimide-cataylzed coupling of a tert-butyloxycarbonylamino acid to the growing peptide attached to a benzhydrylamine resin. The tert-butyloxycarbonyl protecting groups are removed at each stage with trifluoroacetic acid. The nona- or decapeptide is cleaved from the resin and deprotected by use of HF. The crude peptide is purified by the usual techniques, e.g., gel filtration, HPLC and partition chromatography and optionally lyophilization. See also D. H. Coy et al., J. Med. Chem. 19, pages 423-425, (1976).

Typical suitable antiandrogens include non-steroidal antiandrogens such as the imidazolidines, especially 1-(3'-trifluoromethyl-4'-nitrophenyl)-4,4-dimethyl-imidazoline-2,5-dione (also called Anandron) described in U.S. Patent No. 4,097,578, or 4'-nitro-3'-trifluoromethylisobutyranilide (also called flutamide) described in U.S. Patent 4,329,364 as well as the N-(phenylalkanoyl)aniline derivatives disclosed in U.S. Patent 4,386,080 and the 3,4-disubstituted - branched-chain acylanilides disclosed in U.S. Patent 4,239,776 (A.T. Glen et al). Flutamide is the preferred antiandrogen.

Typical suitable steroidal antiandrogens include 6-chloro-1,2-dihydro-17-(acetyloxy)-3'H-cyclopropa[1,2]pregna-1,4,6-triene-3,20-dione, available under the tradename of Androcur from Schering A.G., W. Berlin and 17α-acetyloxy-6-methylpregna-4,6-

diene-3,20-di n , also call d meg strol acetat and available from Mead Johnson & Co., Evansille, Ind. under the tradename of Megace.

Typical suitable antiestrogens include those steroidal and non-steroidal antiestrogens such as (1RS,2RS)-4,4'-diacetoxy-5,5'-difluoro-(1-ethyl-2-methylene)di-m-phenylenediacetate, which is available from Biorex under the tradename of Acefluranol, 6α-chloro-16α-methylpregn-4-ene-3,20-dione which is available from Eli Lilly & Co., Indianapolis, Ind. under the tradename of Clometherone, 6-chloro-17-hydroxypregna-1,4,6-triene-3,20-dione which is available as the acetate salt from Syntex Labs, Palo Alto, Cal. as Delmadione Acetate, 17-hydroxy-6-methyl-19-norpregna-4,6-diene-3,20-dione which is available from Theramex under the name of Lutenyl, 1-[2-[4-[1-(4-methoxyphenyl)-2-nitro-2-phenylethenyl]phenoxy]ethyl]-pyrrolidine which is available as the citrate salt from Parke-Davis Div. of Warner-Lambert Co., Morris Plains, N.J. under the name of Nitromifene Citrate, substituted aminoalkoxyphenylalkenes such as (Z)-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-N,N-dimethylethanamine which is available as the citrate salt from Stuart Pharmaceuticals, Wilmington, Del. as Tamoxifen Citrate (see also Belgian Patent 637,389, March 1964), 3,4-dihydro-2-(p-methoxyphenyl)-1-naphthyl p-[2-(1-pyrrolidinyl)ethoxy]phenyl ketone which is available as the methane sulfonate salt from Eli Lilly & Co. under the tradename of Trioxifene Mesylate, 1-[4'-(2-dimethylaminoethoxy)-phenyl)-1-(3'-hydroxyphenyl)-2-phenyl-but-1-ene, which is available from Klinge Pharma, 6-hydroxy-2-(p-hydroxyphenyl)-benzo(b)thien-3-yl[2-(1-pyrrolidinyl)-ethoxyphenyl]ketone which is available from Eli Lilly & Co. (LY-117018), [6-hydroxy-

2-(4-hydroxyphenyl)b nzo(b)thi n-3-yl]-[4-(2-(1-piperdinyl)-ethoxy)phenyl]methanone, which is available from Eli Lilly & Co. as the hydrogen chloride salt (LY-156758) and meso-3,4-bis(3'-hydroxyphenyl)hexane as well as the dimethyl, dipropyl and 3'-acetoxyphenyl analogues which are described in U.S. Patent 4,094,994 and a series of 1-phenyl-alkane and -alkenes, e.g. (E)-3-cyclopentyl-1-(4-hydroxyphenyl)-1-phenyl-1-butene and 2-cyclopentyl-1-[4-hydroxy-or methoxyphenyl]-3-phenyl-2-propen-1-ol and FC-1157 which is available as the citrate salt from Farmos Group, Ltd., Turku, Finland (see also Eur. Pat. Appln. EP 78,158). It is preferred to use an antiestrogen which shows minimal partial estrogen agonism. FC-1157, LY-117018, LY-156578 and Tamoxifen are the preferred antiestrogens.

The inhibitors of sex steroid biosynthesis found useful in the present invention include those compounds which inhibit biosynthesis of sex steroids and precursor sex steroids of adrenal origin, and/or ovarian (or testicular respectively) preferably of ovarian (or testicular respectively) and adrenal origin.

Typical suitable inhibitors of sex steroid biosynthesis include 3-(4-aminophenyl)-3-ethyl-2,6-piperidinedione which is commonly called aminoglute-thimide, which is an inhibitor of sex steroid biosynthesis of adrenal but also ovarian and testicular origin and which is available from Ciba Pharmaceutical Co., Summit N.J. under tradename Cytadren, or ketoconazole an effective testicular but also adrenal sex steroid biosynthesis inhibitor which is available from Janssen Pharmaceutica, Piscataway, N.J. under the tradename Nizoral.

When an inhibitor of adrenal sex steroid biosynthesis, e.g., aminoglutethimide is administered, cortisol biosynthesis is blocked. Accordingly, a glucocorticoid, e.g. hydrocortisone is administered in physiological amounts sufficient to maintain normal glucocorticoid levels.

In this invention, the LH-RH agonist or antagonist and antiandrogen and, where applicable, the inhibitor of steroid biosynthesis, hydrocortisone and antiestrogen are administered as pharmaceutical compositions via topical, parenteral or oral means. The LH-RH agonist or antagonist is administered parenterally, i.e., intramuscularly, subcutaneously or intravenously by injection or infusion by nasal drops or by suppository, where applicable intra-vaginally. The LH-RH agonist or antagonist also may be microencapsulated in or attached to a biocompatable, biodegradable polymer, e.g., poly(d,l-lactide-co-glycolide) and subcutaneously or intramuscularly injected by a technique called subcutaneous or intramuscular depot to provide continuous, slow release of the LH-RH agonist or antagonist over a period of 30 days or longer. The most preferred route of administration of the LH-RH agonist or antagonist is subcutaneous depot injection. Preferably the antiandrogen will be administered orally. Preferably, the inhibitors of sex steroid biosynthesis such as aminoglutethimide and ketoconazole, and anti-estrogen when used, are administered orally.

The amount of each component administered is determined by the attending clinicians taking into consideration the etiology and severity of the disease, the patient's condition and age, the potency of each component and other factors. In the combination

therapy of sex steroid dependent cancers, such as prostate cancer, breast cancer, testicular cancer, ovarian cancer, colon-rectal cancer, renal cancer, pancreatic cancer, liver cancer, stomach cancer, skin cancer, uterine cancer, brain cancer and larynx cancer especially prostate and breast cancer, according to this invention, the following dosage ranges are suitable.

The LH-RH agonist or antagonist is generally administered at from about 10 to 5000 µg per day, with contemplated dosage ranges of about 10 to 1500 µg per day and about 250 (preferably 200 µg in the therapy of female breast cancer using the combination with sex hormone biosynthesis inhibitor) to 500 µg per day for the LH-RH agonist and to about 50 to 5000 µg per day for the LH-RH antagonist being preferred.

In the most preferred embodiment of this invention, the LH-RH agonist or antagonist is administered subcutaneously in a daily dose of 500 µg for the first 30 days and thereafter subcutaneously in a daily dose of 250 µg regardless of the patients' body weight. When the LH-RH agonist or antagonist is administered, once every 30-day period or even longer, by intramuscular or subcutaneous depot injection, a dose from about 300 to 150,000 µg per 30-day period is used, with a dose of 750 to 15,000 µg per 30-day period being preferred.

The antiandrogen compositions are generally administered in a dosage range of about 0.20 to 40 mg/kg (body weight) per day with 750 mg per day in three equally divided doses being preferred.

The aminoglutethimide compositions (when used) are administered initially in a dosage of 250 mg given at 8-hour intervals and the dosage may be

increas d in incr m nts of 250 mg daily up to a total daily dos of 2 grams.

The ketoconazole compositions (when used) are administered orally in a dose of 250 mg given at 8-hour intervals and may be increased to a daily dose of 2 grams.

Other inhibitors of sex steroid biosynthesis are preferably administered in dosages being equivalent to the dosages given for aminoglutethimide and ketoconazole.

The antiestrogen compositions (when used) are administered in a dosage range of about 0.1 to 10 mg/kg body weight per day, with 10 mg, especially 20 mg, in two equally divided doses being preferred.

The glucocorticoide, especially hydrocortisone compositions (when used) are administered orally in a dosage range of about 0.1 to 20 mg/kg body weight per day. Preferably, the hydrocortisone is administered orally at the dose of about 10 mg in the morning and about 5 mg doses in the afternoon and in the evening.

The LH-RH agonist or antagonist and anti-androgen and inhibitor of sex steroid bisoynthesis and antiestrogen (when used) each may be administered separately or when the modes of administration are the same, all or at least two of them may be administered in the same composition, but in any case the preferred ratio of LH-RH agonist to antiandrogen to antiestrogen (when used) to inhibitor of sex steroid biosynthesis (when used) administered daily will be about 250 µg of LH-RH agonist to about 750 mg of antiandrogen to about 15 mg, especially 20 mg, of anti-estrogen to about 750 mg of inhibitor of sex steroid biosynthesis.

In th th rapy of s x steroid dep ndent canc rs such as, for example, female breast cancer combining the administration of an LH-RH agonist or antagonist, an antiandrogen and an antiestrogen the dosages preferable are as follows: The LH-RH agonist or antagonist is generally administered at from about 10 to 2000 µg per day, with contemplated dosage ranges of 10 to 500 µg per day, 50-250 µg per day and 250 to 500 µg per day being preferred. In the most preferred embodiment of this aspect of the invention, the LH-RH agonist or antagonist is administered subcutaneously in a daily dose of 500 µg for the first 30 days and thereafter subcutaneously in a daily dose of 250 µg regardless of the patients' body weight. When the LH-RH agonist or antagonist is administered, once every 30-day period, by intramuscular or subcutaneous depot injection, a dose from about 300 to 60000 (occasionally 15000) µg per 30-day period is used, with a dose of 750 to 6000 µg per 30-day period being preferred. The antiandrogen compositions are generally administered in a dosage range of about 0.20 to 20 preferably 40 mg/kg (body weight) per day with 375 especially 750 mg per day in three equally divided doses being preferred. The antiestrogen compositions are administered in a dosage range of about 0.1 to 10 mg/kg body weight per day, with 15 mg in three, preferably with 20 mg in two, equally divided doses being preferred. The aminoglutethimide compositions when used are administered initially in a dosage of 250 mg given at 6-hour, preferably 8-hour, intervals and the dosage may be increased in increments of 250 mg daily up to a total daily dose of 2 grams. The ketoconazole

compositions when us d are administered orally in a dose of 200 mg once per day and may be increased to 800 mg once per day.

The LH-RH agonist or antagonist and antiandrogen and antiestrogen each may be administered separately or when the modes of administration are the same, all or two of them may be administered in the same composition, but in any case the preferred ratio of LH-RH agonist to antiandrogen to antiestrogen administered daily will be about 250 µg of LH-RH agonist to about 375 especially 750 mg of antiandrogen to about 15, preferably 20, mg of antiestrogen.

In the therapy of sex steroid dependent cancers, especially such as prostate and breast cancer, according to this invention, it is preferred that the LH-RH agonist is [D-Trp^6,des-Gly NH_2^{10}]LH-RH ethylamide which is administered subcutaneously in single daily dose of 500 µg for the first thirty (30) days of treatment and thereafter in a single daily dose of 250 µg; the antiandrogen is 4'-nitro-3'-trifluoromethyl-isobutyranilide, i.e. flutamide, which is administered orally in three equally divided daily doses of 250 mg each; and the inhibitor of sex steroid biosynthesis is ketoconazole and/or aminoglutethimide, each of which is administered orally in three equally divided doses of 250 mg every 8 hours; and the hydrocortisone (if used) is administered orally at a dose of about 10 mg in the morning and two equally divided doses of about 5 mg, 8 and 16 hours thereafter; and the antiestrogen, when used, is (Z)-2-[p-(1,2-diphenyl-1-butenyl)phenoxy]-N,N-dimethyl ethylamine (Tamoxifen) which is administered orally in two equally divided doses of about 10 mg every 12 hours. In the therapy of, for exampl , female breast cancer using the

combination of th LH-RH agonist, the antiandrog n and antiestrogen discussed here, it is also useful to administer the antiandrogen (flutamide) orally in three equally divided doses of 125, preferably 250 mg.

In the combination therapy of sex steroid dependent cancers according to this invention the administration of the antiandrogen, antiestrogen, inhibitor(s) of steroid biosynthesis, glucocorticoid and LH-RH agonist or LH-RH antagonist can be started in any order of sequence. Preferably the administration of the antiandrogen and/or the antiestrogen is started before (preferably one day before) the administration of the LH-RH agonist or LH-RH antagonist is started. Preferably the administration of the inhibitor(s) of sex steroid biosynthesis is started on the same day as the administration of the LH-RH agonist or LH-RH antagonist. However, the attending clinician may elect to start administration of the LH-RH agonist or antagonist on the first day of treatment.

When patients whose testes or ovaries respectively have already been surgically removed are treated according to this invention, the administration and dosage of the antiandrogen and the other components of the therapy (except the LH-RH agonist or antagonist) are the same as indicated for the therapy in which the LH-RH agonist or antagonist is used.

Normally, an inhibitor of testicular sex steroid biosynthesis such as ketoconazole may be administered to chemically but not surgically castrated patients.

Generally, the inhibitor of testicular sex steroid biosynthesis (when used), e.g., ketoconazole will be administered until the serum levels of T and DHT stimulated by the administration of th LH-RH

agonist are effectively suppressed, normally for one to three weeks. The inhibitor of adrenal sex steroid biosynthesis, e.g., aminoglutethimide may be administered at the start of treatment in the presence or absence of the inhibitor of testicular sex steroid biosynthesis and thereafter continued throughout the period of treatment.

The LH-RH agonists or antagonists useful in the present invention are typically amorphous solids which are freely soluble in water or dilute acids, e.g., HCl, H_2SO_4, citric, acetic, mandelic or fumaric. The LH-RH agonist or antagonist for subcutaneous injection is supplied in vials containing 5 mL of sterile solution with the LH-RH agonist or antagonist at a concentration of about 1.0 mg/mL.

A typical pharmaceutical composition of the LH-RH agonist or antagonist include the LH-RH agonist or antagonist or a pharmaceutically acceptable acid salt thereof, benzyl alcohol, a phosphate buffer (pH=6.9-7.2) and sterile water.

The LH-RH agonist or antagonist for intramuscular or subcutaneous depot injection may be microencapsulated in a biocompatible, biodegradable polymer, e.g., poly (d,l-lactide-co-glycolide) by a phase separation process or formed into a pellet. The microspheres may then be suspended in a carrier to provide an injectable preparation or the depot may be injected in the form of a pellet. See also European Patent Application EPA 58,481 published August 25, 1982 for solid compositions for subdermal injection or implantation or liquid formulations for intramuscular or subcutaneous injections; containing biocompatible,

biodegradeable polym rs such as lactide-glycolide copolymer and an LH-RH agonist, e.g., D-Ser-t-BuO^{6}, Azgly^{10}-LH-RH.

The inhibitors of sex steroid biosynthesis, e.g., aminoglutethimide and ketoconazole and the glucocorticoid, e.g., hydrocortisone (when used) are typically compounded in customary ways for oral administration, e.g., in tablets, capsules and the like.

The antiandrogens useful in the present invention are typically formulated with conventional pharmaceutical excipients, e.g., spray dried lactose and magnesium stearate into tablets or capsules for oral administration. The antiestrogens, when used with the invention, are typically compounded in customary ways for oral administration, e.g., in capsules, tablets, as dragees or even in liquid form, e.g., suspensions or syrups. One or more of the active substances, with or without additional types of active agents, can be worked into tablets or dragee cores by being mixed with solid, pulverulent carrier substances, such as sodium citrate, calcium carbonate or dicalcium phosphate, and binders such as polyvinyl pyrrolidone, gelatin or cellulose derivatives, possibly by adding also lubricants such as magnesium stearate, sodium lauryl sulfate, "Carbowax" or polyethylene glycols. Of course, taste-improving substances can be added in the case of oral-administration forms.

The therapeutically active antiestrogen compound should be present in a concentration of about 0.5-90% by weight of the total mixture, i.e., in amounts that are sufficient for maintaining the above-mentioned dosage range.

As furth r forms of administration, one can use plug capsules, e.g., of hard gelatin, as well as closed soft-gelatin capsules comprising a softener or plasticizer, e.g., glycerine. The plug capsules contain the active substance preferably in the form of granulate, e.g., in mixture with fillers, such as lactose, saccharose, mannitol, starches, such as potato starch or amylopectin, cellulose derivatives or highly-dispersed silicic acids. In soft-gelatin capsules, the active substance is preferably dissolved or suspended in suitable liquids, such as vegetable oils or liquid polyethylene glycols.

In place of oral administration, the active compounds may be administered parenterally. In such case, one can use a solution of the active substance, e.g., in sesame oil or olive oil.

Following the above treatment using the described regimen, tumor growth and bone metastases of sex steroid dependent cancers is inhibited and in some instances complete remission occurs.

The invention also provides kits or single packages combining the pharmaceutical compositions useful for the combination treatment of sex-steroid dependent cancers discussed above. The kits or packages may also contain instructions to use the pharmaceutical compositions in accordance with the present invention. This aspect of the invention is exemplified by the following discussions: For the treatment of female breast cancer a two component kit provides the antiandrogen oral pharmaceutical composition and the LH-RH agonist or LH-RH antagonist parenteral composition or the antiandrogen oral pharmaceutical composition and the antiestrogen oral composition or the oral compositions of the

antiandrogen or antiestrogen and the inhibitor of sex steroid biosynthesis; a three component kit provides the LH-RH agonist or antagonist parenteral pharmaceutical composition, the antiandrogen and the antiestrogen oral pharmaceutical composition, or the oral compositions of the antiandrogen, the antiestrogen and the inhibitor of sex steroid biosynthesis; a four component kit provides the LH-RH agonist or LH-RH antagonist parenteral pharmaceutical composition, the antiandrogen oral pharmaceutical composition, the sex steroid biosynthesis inhibitor oral pharmaceutical composition and the hydrocortisone oral pharmaceutical composition; and a five component kit provides the LH-RH agonist or LH-RH antagonist parenteral pharmaceutical composition, the antiandrogen oral pharmaceutical composition, the antiestrogen oral pharmaceutical composition, the sex steroid biosynthesis inhibitor oral composition and the hydrocortisone oral pharmaceutical composition. Further kits are provided in accordance with the combination therapy of this invention.

For the treatment of prostate cancer, a three component kit provides the antiandrogen oral pharmaceutical composition, the LH-RH agonist or LH-RH antagonist parenteral composition and the sex steroid biosynthesis inhibitor oral pharmaceutical composition; a four component kit provides the LH-RH agonist or LH-RH antagonist parenteral pharmaceutical composition, the antiandrogen oral pharmaceutical composition, the adrenal sex steroid biosynthesis inhibitor oral pharmaceutical composition and the hydrocortisone oral pharmaceutical composition; and a five component kit provides the LH-RH agonist or LH-RH antagonist parenteral pharmaceutical composition, the antiandrogen

oral pharmaceutical composition and th testicular sex st roid biosynthesis inhibitor oral pharmaceutical composition, the adrenal sex steroid biosynthesis inhibitor oral pharmaceutical composition and the hydrocortisone oral pharmaceutical composition. Further kits are provided in accordance with the combination therapy of this invention.

For the treatment of other sex steroid dependent cancers analogous kits are provided according to this invention. The kits may also contain pharmaceutical compositions which contain more than one component of the combination treatment.

The following examples illustrate the invention.

EXAMPLE 1

Figures 1 to 3 show the result of the combination therapy of a male patient having breast cancer according to the invention.

Figure 1 is a bone scintigraph showing multiple bone metastases in a male patient having breast cancer prior to treatment in accordance with an aspect of the present invention.

Figure 2 is a bone scintigraph of the same male patient after treatment for six and a half months in accordance with an aspect of the present invention.

Figure 3 graphically displays serum levels of testicular and adrenal steroids in the male patient treated in accordance with an aspect of the present invention.

Twelve months after modified radical mastectomy with axillary dissection (4 out of 13 positive nodes) in a 66-year old man, bone scintigraphy (Fig. 1) showed multiple bone metastatic lesions in the third and fourth cervical vertebrae, in the first, third and twelth thoracic vertebrae, in the third, fourth and fifth lumbar vertebrae, in both sacro-iliac areas, in both shoulders and in the anterior part of the second right rib. Chest X-Ray and abdominal echogram did not show evidence of additional extension of the disease. The pituitary (LH, FSH and prolactin) as well as steroid [pregnenolone, 17-OH pregnenolone, dehydroepiandrosterone (DHEA), DHEA-sulfate, androst-5-ene-3β,17β-diol(Δ^5-diol), progesterone, 17-OH-progesterone, androstenedione(Δ^4-dione), testosterone (T), dihydrotestosterone (DHT), androstane-3α,17β-diol, androstane-3β,17β-diol, androsterone and cortisol] hormones were within normal limits. Hormone

measurements and calculations w r performed according to known methods. The serum concentration of carcinoembryonic antigen (CEA) as well as a series of laboratory analyses, including complete blood count, sequential multiple analyzer (SMA-12) and urinalysis were normal.

Combined antihormonal treatment with the LH-RH agonist [D-Ser-(tBuO)^6, des-gly-NH$_2$^{10}] LH-RH ethylamide (500 µg daily, s.c.) in combination with the pure antiandrogen Flutamide (250 mg orally every 8h) was started. Six and a half months later bone scintigraphy showed a complete disappearance of increased uptake in all the areas identified 6 1/2 months earlier with no appearance of new lesion (Fig. 2). X-Ray, ultrasonography and clinical examination revealed no sign of any lesion. As illustrated in Fig. 3, the combined treatment caused 95% reduction (compared to control) in serum testosterone (T) and a 25 to 60% fall in the serum levels of adrenal steroids, namely DHEA, DHEAS, Δ^4-dione and Δ^5-diol while not affecting cortisol significantly. Serum levels of DHT and E_2 were also decreased by 20 to 40%. During hormonal therapy, no clinical sign of the disease could be detected. The only side-effects were related to hypoandrogenicity. Thus, after four months of treatment, the patient developed mild climateric-like vasomotor phenomena consisting of perspiration and hot flushes, which did not require treatment. He also complained of loss of libido and sexual potency after two months of treatment.

The present data show a rapid and complete regression of bone metastases in a patient having wide-spread bone metastases from breast cancer. A complete response could b seen within 6 1/2 months after

starting th combined treatment with an LH-RH agonist and a pure antiandrogen.

EXAMPLE 2

INHIBITORY EFFECT OF AMINOGLUTETHIMIDE OR KETOCONAZOLE ON LHRH AGONIST-INDUCED SERUM TESTOSTERONE IN THE DOG

Figures 4 to 6 show the result of the test.

Fig. 4. Serum levels of testosterone during the first 19 days of treatment of dogs with daily administration of 50 µg of [D-Trp^6]LHRH ethylamide.

Fig. 5. Serum levels of testosterone during the first 19 days of treatment of dogs with daily administration to 50 µg of [D-Trp^6]LHRH ethylamide in association with ketoconazole.

Fig. 6. Serum levels of testosterone during the first 19 days of treatment of dogs with daily administration of 50 µg of [D-Trp^6]LHRH ethylamide in association with aminoglutethimide.

While LHRH agonists achieve castration levels of androgens during chronic treatment in men, the first 5 to 10 days of administration of the peptide are associated with increased serum levels of testosterone and dihydrotestosterone (Labrie et al., J. Androl. 1: 209-228, 1980). These increased levels of testicular androgens are accompanied by an exacerbation of the symptoms and signs of prostate cancer in significant proportion of cases (Kahan et al., The Lancet i, 971-972, 1984).

The following experiment shows that the LHRH agonist-induced rise in serum testosterone can be prevented by simultaneous administration of the steroid

biosynthesis inhibitors aminoglutethimide or ketoconazole.

Eighteen mongrel dogs weighing between 20-30 kg were randomly divided into three groups. The animals were housed one per cage and fed Purina Dog Chow and tap water ad libitum.

The animals of group 1 were injected daily (at 0700h) with 50 µg of the LHRH agonist [D-Trp^6, des-Gly-NH$_2$^{10}]LHRH ethylamide (LHRH-A). In group 2, the animals were also injected daily with 50 µg of LHRH-A and took in addition 200 mg of ketoconazole per os at 0700h, 1500h and 2300h. The animals of group 3 received daily 50 µg of LHRH-A and 125 mg of aminoglutethimide per os at 0700h, 1500h, 2300h plus 10 mg of hydrocortisone twice daily.

Blood samples were collected at 0700h, 0900h, 1100h and 1500h one day before treatment and every day during the treatment. Plasma was extracted with diethyl ether and testosterone was separated on LH-20 columns before RIA measurements.

Fig. 4 shows that on one to six days following the start of treatment with the daily injection of 50 µg of the LHRH agonist [D-Trp^6]LHRH ethylamide, there is an increase in serum testosterone up to approximately 500% above control. Thereafter, there is a decrease of the level of this androgen which reaches near-castration levels on day 19. It can be seen in Fig. 5 and 6 that the simultaneous administration of the steroid inhibitors ketoconazole or aminoglutethimide almost completely prevented this rise in serum testosterone which occur during the first days of treatment with the peptide.

These data clearly indicate the usefulness of the combined administration of inhibitors of steroid

biosynth sis in order to prev nt th rise of testicular serum androgens during the first days of treatment with LHRH agonists.

Since dogs do not have adrenal production of androgens the influence of the administration of an antiandrogen in combination with the administration of the LH-RH agonist and the inhibitors of sex steroid biosynthesis cannot be demonstrated in this species. However, it can be concluded from the test results that in men the administration of an antiandrogen (especially flutamide) in combination with the LH-RH agonist or antagonist and the inhibitors of sex steroid biosynthesis which inhibit the secretion of precursor adrenal androgens substantially or completely decreases the content of androgens in the system and thus contributes to the inhibition of androgen sensitive cancer growth.

The important aspects of this invention can be summarized as follows:

The invention provides:

1) A method of treating sex steroid dependent cancers in a warm-blooded animal which comprises administering a pharmaceutically effective amount of an antiandrogen and/or an antiestrogen and/or at least one inhibitor of sex steroid biosynthesis to the animal whose hormone output of the testes or ovaries, is blocked, with the proviso that the method of treating prostate cancer comprises administering pharmaceutically effective amounts of an antiandrogen and at least one inhibitor of sex steroid biosynthesis and the method of treating breast cancer comprises administering a pharmaceutically effective amount (1) of an antiandrogen or (2) of at least one inhibitor of s x steroid biosynthesis

or (3) of an antiandrog n and an antiestrogen or (4) of an antiandrogen and at least one inhibitor of sex steroid biosynthesis or (5) of an antiestrogen and at least one inhibitor of sex steroid biosynthesis or (6) of an antiandrogen and an antiestrogen and at least one inhibitor of sex steroid biosynthesis.

2) A kit comprising a combination of pharmaceutical compositions for combination therapy of sex steriod dependent cancers in a warm-blooded animal whose hormone output of the testes or ovaries, is blocked, the combination consisting of pharmaceutical compositions comprising (1) an antiandrogen and an antiestrogen or (2) an antiandrogen and at least one inhibitor of sex steroid biosynthesis or (3) an antiestrogen and at least one inhibitor of sex steroid biosynthesis or (4) an antiandrogen and an antiestrogen and at least one inhibitor of sex steroid biosynthesis.

3) A kit comprising a pharmaceutical composition comprising an antiandrogen and/or a pharmaceutical composition comprising an antiestrogen and/or one or more pharmaceutical composition(s) comprising an inhibitor of sex steroid biosynthesis together with instructions for using the composition(s) or each of these compositions for the treatment of sex steroid dependent cancers in a warm-blooded animal by a combination therapy involving administering a pharmaceutically effective amount of an antiandrogen and/or an antiestrogen and/or at least one inhibitor of sex steroid biosynthesis to the animal whose hormone output of the testes or ovaries, is blocked, with the proviso that the instructions for treating prostate cancer recomm nd administ ring pharmac utically

effectiv amounts of an antiandrogen and at least one inhibitor of sex steroid biosynthesis and the instructions for treating breast cancer recommend administering a pharmaceutically effective amount (1) of an antiandrogen or (2) of at least one inhibitor of sex steroid biosynthesis or (3) of an antiandrogen and an antiestrogen or (4) of an antiandrogen and at least one inhibitor of sex steroid biosynthesis or (5) of an antiestrogen and at least one inhibitor of sex steroid biosynthesis or (6) of an antiandrogen and an antiestrogen and at least one inhibitor of sex steroid biosynthesis.

4) Use of an antiandrogen and/or an antiestrogen and/or at least one inhibitor of sex steroid biosynthesis for treating sex steroid dependent cancers in a warm-blooded animal by administering a pharmaceutically effective amount of an antiandrogen and/or an antiestrogen and/or at least one inhibitor of sex steroid biosynthesis to the animal whose hormone output of the testes or ovaries is blocked with the proviso that treating prostate cancer comprises administering pharmaceutically effective amounts of an antiandrogen and at least one inhibitor of sex steroid biosynthesis and treating breast cancer comprises administering a pharmaceutically effective amount (1) of an antiandrogen or (2) of at least one inhibitor of sex steroid biosynthesis or (3) of an antiandrogen and an antiestrogen or (4) of an antiandrogen and at least one inhibitor of sex steroid biosynthesis or (5) of an antiestrogen and at least one inhibitor of sex steroid biosynthesis or (6) of an antiandrogen and an antiestrogen and at least one inhibitor of sex steroid biosynthesis.

5) The use of an antiandrogen and/or an antiestrogen and/or at least one inhibitor of sex steroid biosynthesis for the manufacture of a medicament for combination therapy of sex steroid dependent cancers in a warm-blooded animal which therapy comprises administering a pharmaceutically effective amount of an antiandrogen and/or an antiestrogen and/or at least one inhibitor of sex steroid biosynthesis to the animal whose hormone output of the testes or ovaries is blocked, with the proviso that the therapy of prostate cancer comprises administering pharmaceutically effective amounts of an antiandrogen and at least one inhibitor of sex steroid biosynthesis and the therapy of breast cancer comprises administering a pharmaceutically effective amount (1) of an antiandrogen or (2) of at least one inhibitor of sex steroid biosynthesis or (3) of an antiandrogen and an antiestrogen or (4) of an antiandrogen and at least one inhibitor of sex steroid biosynthesis or (5) of an antiestrogen and at least one inhibitor of sex steroid biosynthesis or (6) of an antiandrogen and an antiestrogen and at least one inhibitor of sex steroid biosynthesis.

6) A combination of pharmaceutical compositions for combination therapy of sex steroid dependent cancers in a warm-blooded animal whose hormone output of the testes or ovaries is blocked, the combination consisting of pharmaceutical compositions comprising an antiandrogen and antiestrogen or an antiandrogen and at least one inhibitor of sex steroid biosynthesis or an < > antiandrogen and an antiestrogen and at least one inhibitor of sex steroid biosynthesis.

<antiestrogen and at least one inhibitor of sex steroid biosynthesis or an>

Preferred aspects of the inv ntion can b summarized as follows:

a) For treating testicular cancer, ovarian cancer, colon-rectal cancer, renal cancer, pancreatic cancer, liver cancer, stomach cancer, skin cancer, uterine cancer, brain cancer and larynx cancer: administering an antiandrogen and/or an antiestrogen and/or at least one inhibitor of sex steroid biosynthesis.

b) For treating breast cancer, testicular cancer, ovarian cancer, colon-rectal cancer, renal cancer, pancreatic cancer, liver cancer, stomach cancer, skin cancer, uterine cancer, brain cancer and larynx cancer: administration of an antiandrogen or of an antiandrogen and an antiestrogen or of an antiandrogen and at least one inhibitor of sex steroid biosynthesis or of an antiestrogen and at least one inhibitor of sex steroid biosynthesis or of an antiandrogen and an antiestrogen and at least one inhibitor of sex steroid biosynthesis.

c) Administration of the antiandrogen and/or the antiestrogen and/or at least one inhibitor of sex steroid biosynthesis and, if used, the glucocorticoid, such as hydrocortisone, to an animal whose hormone output of the testes or ovaries, has been blocked by surgical removal thereof.

d) Administration of the antiandrogen and/or the antiestrogen and/ or at least one inhibitor of sex steroid biosynthesis and, if used, the glucocorticoid, such as hydrocortisone to an animal whose hormone output of the testes or ovaries, is blocked by chemical

m ans such as by an LH-RH agonist or LH-RH antagonist of which a pharmaceutically effective amount is administered to the animal in association with the antiandrogen and/or the antiestrogen and/or the inhibitor(s) of steroid biosynthesis and/or the glucocorticoid.

e) Administration of the antiandrogen, the antiestrogen, the inhibitor(s) of sex steroid biosynthesis, the LH-RH agonist or LH-RH antagonist and the glucocorticoid (if applicable) in the form of individual compositions.

f) Preferably the antiandrogen and/or the antiestrogen is started one day before the administration of the LH-RH agonist or LH-RH antagonist and of the inhibitor(s) of sex steroid biosynthesis (if used) and the glucocorticoid (if used).

g) Administration of the LH-RH agonist or LH-RH antagonist in the form of a parenteral composition.

h) Administration of the antiandrogen, antiestrogen, the inhibitor(s) of sex steroid biosynthesis and the glucocorticoid such as the hydrocortisone in the form of oral compositions.

i) The preferred antiandrogen is 4'-nitro-3'-trifluoromethylisobutyranilide or 1-(3'-trifluoromethyl-4'-nitrophenyl)-4,4-dimethylimidazoline-2,5-dione.

j) The preferred LH-RH agonist is a nonapeptide or a decapeptide represented by the formula : L-pyro-glutamyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-X-Y-

L-arginyl-L-prolyl-Z, wh rein X is D-tryptophyl, D-leucyl, D-alanyl, iminobenzyl-D-histidyl, 3-(2-naphthyl)-D-alanyl, O-tert-butyl-D-seryl, D-tyrosyl, D-lysyl, D-phenylalanyl or N-methyl-D-alanyl and wherein Y is L-leucyl, N^{α}-methyl-L-leucyl, D-leucyl, N^{α}-methyl-D-leucyl, or D-alanyl and wherein Z is glycyl-NHR_1 or NHR_1 wherein R_1 is H, lower alkyl or haloloweralkyl.

k) Preferably aminoglutethimide and/or ketokonazole is/are used as inhibitors of sex steroid biosynthesis.

l) The preferred antiestrogen is (Z)-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-N,N-dimethylethanamine or LY-117018.

Relating to these preferred aspects the invention provides kits comprising compositions containing the relevant active substances, possibly together with instructions to use them.

Claims:

1) A method of treating sex steroid dependent cancers in a warm-blooded animal which comprises administering a pharmaceutically effective amount of an antiandrogen and/or an antiestrogen and/or at least one inhibitor of sex steroid biosynthesis to the animal whose hormone output of the testes or ovaries, is blocked, with the proviso that the method of treating prostate cancer comprises administering pharmaceutically effective amounts of an antiandrogen and at least one inhibitor of sex steroid biosynthesis and the method of treating breast cancer comprises administering a pharmaceutically effective amount (1) of an antiandrogen or (2) of at least one inhibitor of sex steroid biosynthesis or (3) of an antiandrogen and an antiestrogen or (4) of an antiandrogen and at least one inhibitor of sex steroid biosynthesis or (5) of an antiestrogen and at least one inhibitor of sex steroid biosynthesis or (6) of an antiandrogen and an antiestrogen and at least one inhibitor of sex steroid biosynthesis.

2) The method according to claim 1 which for treating testicular cancer, ovarian cancer, colon-rectal cancer, renal cancer, pancreatic cancer, liver cancer, stomach cancer, skin cancer, uterine cancer, brain cancer and larynx cancer comprises administering an antiandrogen and/or an antiestrogen and/or at least one inhibitor of sex steroid biosynthesis.

3) The method according to claim 1 or 2 which for treating breast cancer, testicular cancer, ovarian cancer, colon-rectal cancer, renal cancer, pancreatic cancer, liver cancer, stomach cancer, skin cancer, uterine cancer, brain cancer and larynx cancer comprises administration of an antiandrogen or of an antiandrogen and an antiestrogen or of an antiandrogen and at least one inhibitor of sex steroid biosynthesis or of an antiestrogen and at least one inhibitor of sex steroid biosynthesis or of an antiandrogen and an antiestrogen and at least one inhibitor of sex steroid biosynthesis.

4) The method according to any one of claims 1 to 3 which further comprises administration of a glucocorticoid such as, for example, hydrocortisone.

5) The method according to any one of claims 1 to 4 wherein the antiandrogen and/or the antiestrogen and/or at least one inhibitor of sex steroid biosynthesis and, if used, the glucocorticoid, such as hydrocortisone, is/are administered to an animal whose hormone output of the testes or ovaries, has been blocked by surgical removal thereof.

6) The method according to any one of claims 1 to 4 wherein the antiandrogen and/or the antiestrogen and/or at least one inhibitor of sex steroid biosynthesis and, if used, the glucocorticoid, such as hydrocortisone is/are administered to an animal whose hormone output of the testes or ovaries, is blocked by chemical means such as by an LH-RH agonist or LH-RH antagonist of which a pharmac utically ffective amount

is administ red to th animal in association with th antiandrogen and/or the antiestrogen and/or the inhibitor(s) of steroid biosynthesis and/or the glucocorticoid.

7) The method according to any one of claims 1 to 6 which comprises administering the antiandrogen, the antiestrogen, the inhibitor(s) of sex steroid biosynthesis, the LH-RH agonist or LH-RH antagonist and the glucocorticoid (if applicable) in the form of individual compositions.

8) The method according to claim 6 or 7 wherein the administration of the antiandrogen and/or the antiestrogen is started one day before the administration of the LH-RH agonist or LH-RH antagonist and of the inhibitor(s) of sex steroid biosynthesis (if used) and the glucocorticoid (if used).

9) The method according to any one of claims 6 to 8 which comprises administering the LH-RH agonist or LH-RH antagonist in the form of a parenteral composition.

10) The method according to any one of claims 1 to 9 which comprises administering the antiandrogen, antiestrogen, the inhibitor(s) of sex steroid biosynthesis and the glucocorticoid such as the hydrocortisone in the form of oral compositions.

11) The method according to any one of claims 1 to 10 wherein the antiandrogen is 4'-nitro-3'-trifluoro-methylisobutyranilide or 1-(3'-trifluoromethyl-4'-nitrophenyl)-4,4-dimethylimidazoline-2,5-dione.

12) Th method according to any one of claims 1 to 4 and 6 to 11 wherein the LH-RH agonist is a nonapeptide or a decapeptide represented by the formula : L-pyroglutamyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-X-Y-L-arginyl-L-prolyl-Z, wherein X is D-tryptophyl, D-leucyl, D-alanyl, iminobenzyl-D-histidyl, 3-(2-naphthyl)-D-alanyl, O-tert-butyl-D-seryl, D-tyrosyl, D-lysyl, D-phenylalanyl or N-methyl-D-alanyl and wherein Y is L-leucyl, N^{α}-methyl-L-leucyl, D-leucyl, N^{α}-methyl-D-leucyl, or D-alanyl and wherein Z is glycyl-NHR_1 or NHR_1 wherein R_1 is H, lower alkyl or haloloweralkyl.

13) The method according to any one of claims 1 to 12 wherein aminoglutethimide and/or ketokonazole are used as inhibitors of sex steroid biosynthesis.

14) The method according to any one of claims 1 to 13 wherein the antiestrogen is (Z)-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-N,N-dimethylethanamine or LY-117018.

15) A kit comprising a combination of pharmaceutical compositions for combination therapy of sex steriod dependent cancers in a warm-blooded animal whose hormone output of the testes or ovaries, is blocked, the combination consisting of pharmaceutical compositions comprising (1) an antiandrogen and an antiestrogen or (2) an antiandrogen and at least one inhibitor of sex steroid biosynthesis or (3) an antiestrogen and at least one inhibitor of sex steroid biosynthesis or (4) an antiandrogen and an antiestrogen and at least one inhibitor of sex steroid biosynthesis.

16) Th kit according to claim 15 which further compris s a pharmac utical composition comprising a LH-RH agonist or LH-RH antagonist.

17) The kit according to claim 15 or 16 which further comprises a pharmaceutical composition comprising a glucocorticord.

18) The kit according to any one of claims 15 to 17 which for the combination therapy of prostate cancer comprises a pharmaceutical composition comprising an antiandrogen together with at least one pharmaceutical composition comprising an inhibitor of sex steroid biosynthesis.

19) The kit according to any one of claims 15 to 18 wherein the composition comprising the LH-RH agonist or LH-RH antagonist is in the form of a parenteral composition.

20) The kit according to any one of claims 15 to 19 wherein the composition comprising the antiandrogen and/or the composition comprising the antiestrogen and/or the composition comprising the glucocorticord and/or the composition(s) comprising an inhibitor of sex steroid biosynthesis is (are) in the form of an oral composition.

21) The kit according to any one of claims 15 to 20 wherein the antiandrogen is 4'-nitro-3'-trifluoro-methylisobutyranilide or 1-(3'-trifluoromethyl-4'-nitrophenyl)-4,4-dimethylimidazoline-2,5-dione.

22) Th kit according to any on of claims 16 to 21 wh r in th LH-RH agonist is a nonap ptid or a decapeptide represented by the formula: L-pyroglutamyl-L-histidyl-l-tryptophyl-L-seryl-L-tyrosyl-X-Y-L-arginyl-L-prolyl-Z, wherein X is D-tryptophyl, D-leucyl, D-alanyl, O-tert-butyl-D-seryl, D-tyrosyl, D-lysyl, D-phenylalanyl or N-methyl-D-alanyl and wherein Y is L-leucyl, Nα-methyl-L-leucyl, D-leucyl, N^{α}-methyl-D-leucyl, or D-alanyl and wherein Z is glycyl-NHR_1 or NHR_1 wherein R_1 is H, lower alkyl or haloloweralkyl.

23). The kit according to any one of claims 15 to 22 comprising aminoglutethimide and/or ketokonazole as inhibitors of sex steroid biosynthesis.

24) The kit according to any one of claims 15 to 23 wherein the antiestrogen is (Z)-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-N,N-dimethylethanamine or LY-117018.

25) .A kit comprising a pharmaceutical composition comprising an antiandrogen and/or a pharmaceutical composition comprising an antiestrogen and/or one or more pharmaceutical composition(s) comprising an inhibitor of sex steroid biosynthesis together with instructions for using the composition(s) or each of these compositions for the treatment of sex steroid dependent cancers in a warm-blooded animal by a combination therapy involving administering a pharmaceutically effective amount of an antiandrogen and/or an antiestrogen and/or at least one inhibitor of sex steroid biosynthesis to the animal whose hormone output of the testes or ovaries, is blocked, with the proviso that the instructions for treating prostate cancer recommend administering pharmac utically

effective amounts of an antiandrogen and at least one inhibitor of sex steroid biosynthesis and the instructions for treating breast cancer recommend administering a pharmaceutically effective amount (1) of an antiandrogen or (2) of at least one inhibitor of sex steroid biosynthesis or (3) of an antiandrogen and an antiestrogen or (4) of an antiandrogen and at least one inhibitor of sex steroid biosynthesis or (5) of an antiestrogen and at least one inhibitor of sex steroid biosynthesis or (6) of an antiandrogen and an antiestrogen and at least one inhibitor of sex steroid biosynthesis.

26) The kit according to claim 25 containing instructions which for treating testicular cancer, ovarian cancer, colon-rectal cancer, renal cancer, pancreatic cancer, liver cancer, stomach cancer, skin cancer, uterine cancer, brain cancer and larynx cancer recommend administering an antiandrogen and/or an antiestrogen and/or at least one inhibitor of sex steroid biosynthesis.

27) The kit according to claim 25 or 26 containing instructions which for treating breast cancer, testicular cancer, ovarian cancer, colon-rectal cancer, renal cancer, pancreatic cancer, liver cancer, stomach cancer, skin cancer, uterine cancer, brain cancer and larynx cancer recommend the administration of (1) an antiandrogen or (2) of an antiandrogen and an antiestrogen or (3) of an antiandrogen and at least one inhibitor of sex steroid biosynthesis or (4) of an antiestrogen and at least one inhibitor of sex steroid

biosynth sis or (5) of an antiandrog n and an antiestrogen and at least one inhibitor of sex steroid biosynthesis.

28) The kit according to any one of claims 25 to 27 which further comprises a glucocorticoid such as, for example, hydrocortisone.

29) The kit according to any one of claims 25 to 28 containing instructions which recommend that the anti-androgen and/or the antiestrogen and/or at least one inhibitor of sex steroid biosynthesis and, if used, the glucocorticoid, such as hydrocortisone, is/are administered to an animal whose hormone output of the testes or ovaries has been blocked by surgical removal thereof.

30) The kit according to any one of claims 25 to 28 containing instructions which recommend that the antiandrogen and/or the antiestrogen and/ or at least one inhibitor of sex steroid biosynthesis and, if used, the glucocorticoid, such as hydrocortisone is/are administered to an animal whose hormone output of the testes or ovaries, is blocked by chemical means such as by an LH-RH agonist or LH-RH antagonist of which a pharmaceutically effective amount is administered to the animal in association with the antiandrogen and/or the antiestrogen and/or the inhibitor(s) of steroid biosynthesis and/or the glucocorticoid.

31) The kit according to any one of claims 25 to 30 comprising the antiandrogen, the antiestrogen, the

inhibitor(s) of s x steroid biosynthesis, th LH-RH agonist or LH-RH antagonist and the glucocorticoid (if applicable) in the form of individual compositions.

32) The kit according to claim 30 or 31 containing instructions which recommend that the administration of the antiandrogen and/or the antiestrogen is started one day before the administration of the LH-RH agonist or LH-RH antagonist and of the inhibitor(s) of sex steroid biosynthesis (if used) and the glucocorticoid (if used).

33) The kit according to any one of claims 30 to 32 which comprises the LH-RH agonist or LH-RH antagonist in the form of a parenteral composition.

34) The kit according to any one of claims 25 to 33 which comprises the antiandrogen, the antiestrogen, the inhibitor(s) of sex steroid biosynthesis and the glucocorticoid such as the hydrocortisone in the form of oral compositions.

35) The kit according to any one of claims 25 to 34 wherein the antiandrogen is 4'-nitro-3'-trifluoro-methylisobutyranilide or 1-(3'-trifluoromethyl-4'-nitrophenyl)-4,4-dimethylimidazoline-2,5-dione.

36) The kit according to any one of claims 25 to 28 and 30 to 35 wherein the LH-RH agonist is a nonapeptide or a decapeptide represented by the formula : L-pyroglutamyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-X-Y-L-arginyl-L-prolyl-Z, wherein X is D-tryptophyl, D-leucyl, D-alanyl, iminobenzyl-D-histidyl, 3-(2-naphthyl)-D-alanyl, O-tert-butyl-D-s ryl, D-tyrosyl, D-

lysyl, D-phenylalanyl or N-methyl-D-alanyl and wherein Y is L-leucyl, N^{α}-methyl-L-leucyl, D-leucyl, N^{α}-methyl-D-leucyl, or D-alanyl and wherein Z is glycyl-NHR_1 or NHR_1 wherein R_1 is H, lower alkyl or haloloweralkyl.

37) The kit according to any one of claims 25 to 36 wherein the inhibitor(s) of sex steroid biosynthesis is/are aminoglutethimide and/or ketokonazole.

38) The kit according to any one of claims 25 to 37 wherein the antiestrogen is (Z)-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-N,N-dimethylethanamine or LY-117018.

39) Use of an antiandrogen and/or an antiestrogen and/or at least one inhibitor of sex steroid biosynthesis for treating sex steroid dependent cancers in a warm-blooded animal by administering a pharmaceutically effective amount of an antiandrogen and/or an antiestrogen and/or at least one inhibitor of sex steroid biosynthesis to the animal whose hormone output of the testes or ovaries is blocked with the proviso that treating prostate cancer comprises administering pharmaceutically effective amounts of an antiandrogen and at least one inhibitor of sex steroid biosynthesis and treating breast cancer comprises administering a pharmaceutically effective amount (1) of an antiandrogen or (2) of at least one inhibitor of sex steroid biosynthesis or (3) of an antiandrogen and an antiestrogen or (4) of an antiandrogen and at least one inhibitor of sex steroid biosynthesis or (5) of an antiestrogen and at least one inhibitor of sex steroid biosynthesis or (6) of an antiandrogen and an antiestrogen and at least one inhibitor of sex steroid biosynthesis.

40) Th use according to claim 39 which for treating testicular cancer, ovarian cancer, colon-rectal cancer, renal cancer, pancreatic cancer, liver cancer, stomach cancer, skin cancer, uterine cancer, brain cancer and larynx cancer comprises administering an antiandrogen and/or an antiestrogen and/or at least one inhibitor of sex steroid biosynthesis.

41) The use according to claim 39 or 40 which for treating breast cancer, testicular cancer, ovarian cancer, colon-rectal cancer, renal cancer, pancreatic cancer, liver cancer, stomach cancer, skin cancer, uterine cancer, brain cancer and larynx cancer comprises administration of an antiandrogen or of an antiandrogen and an antiestrogen or of an antiandrogen and at least one inhibitor of sex steroid biosynthesis or of an antiestrogen and at least one inhibitor of sex steroid biosynthesis or of an antiandrogen and an antiestrogen and at least one inhibitor of sex steroid biosynthesis.

42) The use according to any one of claims 39 to 41 which further comprises administration of a glucocorticoid such as, for example, hydrocortisone.

43) The use according to any one of claims 39 to 41 according to which the antiandrogen and/or the antiestrogen and/or at least one inhibitor of sex steroid biosynthesis and, if used, the glucocorticoid, such as hydrocortisone, is/are administered to an animal whose hormone output of the testes or ovaries, has been blocked by surgical removal thereof.

44) Th us according to any on of claims 39 to 42 according to which the antiandrogen and/or the antiestrogen and/ or at least one inhibitor of sex steroid biosynthesis and, if used, the glucocorticoid, such as hydrocortisone is/are administered to an animal whose hormone output of the testes or ovaries, is blocked by chemical means such as by an LH-RH agonist or LH-RH antagonist of which a pharmaceutically effective amount is administered to the animal in association with the antiandrogen and/or the antiestrogen and/or the inhibitor(s) of steroid biosynthesis and/or the glucocorticoid.

45) The use according to any one of claims 39 to 44 which comprises administering the antiandrogen, the antiestrogen, the inhibitor(s) of sex steroid biosynthesis, the LH-RH agonist or LH-RH antagonist and the glucocorticoide (if applicable) in the form of individual compositions.

46) The use according to claim 44 or 45 wherein the administration of the antiandrogen and/or the antiestrogen is started one day before the administration of the LH-RH agonist or LH-RH antagonist and of the inhibitor(s) of sex steroid biosynthesis (if used) and the glucocorticoid (if used).

47) The use according to any one of claims 44 to 46 which comprises administering the LH-RH agonist or LH-RH antagonist in the form of a parenteral composition.

48) The use according to any one of claims 44 to 47 which comprises administering the antiandrogen, the antiestrogen, the inhibitor(s) of sex steroid

biosynth sis and the glucocorticoid such as th hydrocortisone in the form of oral compositions.

49) The use according to any one of claims 44 to 48 wherein the antiandrogen is 4'-nitro-3'-trifluoro-methylisobutyranilide or 1-(3'-trifluoromethyl-4'-nitrophenyl)-4,4-dimethylimidazoline-2,5-dione.

50) The use according to any one of claims 44 to 49 wherein the LH-RH agonist is a nonapeptide or a decapeptide represented by the formula : L-pyroglutamyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-X-Y-L-arginyl-L-prolyl-Z, wherein X is D-tryptophyl, D-leucyl, D-alanyl, iminobenzyl-D-histidyl, 3-(2-naphthyl)-D-alanyl, O-tert-butyl-D-seryl, D-tyrosyl, D-lysyl, D-phenylalanyl or N-methyl-D-alanyl and wherein Y is L-leucyl, N^{α}-methyl-L-leucyl, D-leucyl, N^{α}-methyl-D-leucyl, or D-alanyl and wherein Z is glycyl-NHR_1 or NHR_1 wherein R_1 is H, lower alkyl or haloloweralkyl.

51) The use according to any one of claims 39 to 50 wherein aminoglutethimide and/or ketokonazole are used as inhibitors of sex steroid biosynthesis.

52) The use according to any one of claims 39 to 51 wherein the antiestrogen is (Z)-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-N,N-dimethylethanamine or LY-117018.

53) The use of an antiandrogen and/or an antiestrogen and/or at least one inhibitor of sex steroid biosynthesis for the manufacture of a medicament for combination therapy of sex steroid dependent cancers in a warm-blooded animal which therapy comprises administering a pharmaceutically effective amount of an

antiandrog n and/or an antiestrog n and/or at least one inhibitor of sex steroid biosynthesis to the animal whose hormone output of the testes or ovaries is blocked, with the proviso that the therapy of prostate cancer comprises administering pharmaceutically effective amounts of an antiandrogen and at least one inhibitor of sex steroid biosynthesis and the therapy of breast cancer comprises administering a pharmaceutically effective amount (1) of an antiandrogen or (2) of at least one inhibitor of sex steroid biosynthesis or (3) of an antiandrogen and an antiestrogen or (4) of an antiandrogen and at least one inhibitor of sex steroid biosynthesis or (5) of an antiestrogen and at least one inhibitor of sex steroid biosynthesis or (6) of an antiandrogen and an antiestrogen and at least one inhibitor of sex steroid biosynthesis.

54) The use of an antiandrogen and/or an antiestrogen and/or at least one inhibitor of sex steroid biosynthesis according to claim 53 for the manufacture of a medicament for combination therapy of testicular cancer, ovarian cancer, colon-rectal cancer, renal cancer, pancreatic cancer, liver cancer, stomach cancer, skin cancer, uterine cancer, brain cancer and larynx cancer.

55) The use of an antiandrogen or of an antiandrogen and an antiestrogen or of an antiandrogen and at least one inhibitor of sex steroid biosynthesis or of an antiestrogen and at least one inhibitor of sex steroid biosynthesis or of an antiandrogen and an antiestrogen and at least one inhibitor of sex steroid biosynthesis according to claim 53 or 54 for the manufacture of a medicament for combination therapy of br ast canc r,

t sticular cancer, ovarian canc r, colon-rectal cancer, renal cancer, pancreatic cancer, liver cancer, stomach cancer, skin cancer, uterine cancer, brain cancer and larynx cancer.

56) The use according to any one of claims 53 to 55 which further comprises administration of a glucocorticoid such as, for example, hydrocortisone.

57) The use according to any one of claims 53 to 56 which further comprises useof a LH-RH agonist or LH-RH antagonist.

58) The use according to any one of claims 53 to 57 wherein the antiandrogen is 4'-nitro-3'-trifluoromethylisobutyranilide or 1-(3'-trifluoromethyl-4'-nitrophenyl)-4,4-dimethylimidazoline-2,5-dione.

59) The use according to claim 57 or 58 wherein the LH-RH agonist is a nonapeptide or a decapeptide represented by the formula : L-pyroglutamyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-X-Y-L-arginyl-L-prolyl-Z, wherein X is D-tryptophyl, D-leucyl, D-alanyl, iminobenzyl-D-histidyl, 3-(2-naphthyl)-D-alanyl, O-tert-butyl-D-seryl, D-tyrosyl, D-lysyl, D-phenylalanyl or N-methyl-D-alanyl and wherein Y is L-leucyl, N^{α}-methyl-L-leucyl, D-leucyl, N^{α}-methyl-D-leucyl, or D-alanyl and wherein Z is glycyl-NHR_1 or NHR_1 wherein R_1 is H, lower alkyl or haloloweralkyl.

60) The use according to any one of claims 53 to 59 wherein aminoglutethimide and/or ketokonazole are used as inhibitors of sex steroid biosynthesis.

61) Th us according to any one of claims 57 to 60 wherein the antiestrogen is (Z)-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-N,N-dimethylethanamine or LY-117018.

62) A combination of pharmaceutical compositions for combination therapy of sex steroid dependent cancers in a warm-blooded animal whose hormkone output of the testes or ovaries is blocked, the combination consisting of pharmaceutical compositions comprising an antiandrogen and an antiestrogen or an antiandrogen and at least one inhibitor of sex steroid biosynthesis or of an antiandrogen and an antiestrogen and at least one inhibitor of sex steroid biosynthesis.

63) The combination according to claim 62 which further comprises a pharmaceutical composition comprising a LH-RH agonist or LH-RH antagonist.

64) The combination according to claim 62 or 63 which further comprises a pharmaceutical composition comprising a glucocorticoid, preferably hydrocortisone.

65) The combination according to any one of claims 62 to 64 for treating breast cancer, testicular cancer, ovarian cancer, colon-rectal cancer, renal cancer, pancreatic cancer, liver cancer, stomach cancer, skin cancer, uterine cancer, brain cancer and larynx cancer.

66) The combination according to any one of claims 62 to 65 consisting of pharmaceutical compositions comprising an antiandrogen and at least one inhibitor of sex steroid biosynthesis and if desired an LH-RH agonist or antagonist and if desired an glucocorticord for treating prostate canc r.

67) The combination according to claim 62 for combination therapy of sex steroid dependent cancers of an animal whose hormone output of the testes or ovaries has been blocked by surgical removal thereof.

68) The combination according claim 66 which comprises the LH-RH agonist or LH-RH antagonist in the form of a parenteral composition.

69) The combination according to any one of claims 62 to 68 which comprises the antiandrogen, the anti-estrogen, the inhibitor(s) of sex steroid biosynthesis and the glucocorticoid such as hydrocortisone in the form of oral compositions.

70) The combination according to any one of claims 62 to 69 wherein the antiandrogen is 4'-nitro-3'-trifluoromethylisobutyranilide or 1-(3'-trifluoromethyl-4'-nitrophenyl)-4,4-dimethylimidazoline-2,5-dione.

71) The combination according to any one of claims 63 to 66 and 68 to 70 wherein the LH-RH agonist is a nonapeptide or a decapeptide represented by the formula : L-pyroglutamyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-X-Y-L-arginyl-L-prolyl-Z, wherein X is D-tryptophyl, D-leucyl, D-alanyl, iminobenzyl-D-histidyl, 3-(2-naphthyl)-D-alanyl, O-tert-butyl-D-seryl, D-tyrosyl, D-lysyl, D-phenylalanyl or N-methyl-D-alanyl and wherein Y is L-leucyl, N^{α}-methyl-L-leucyl, D-leucyl, N^{α}-methyl-D-leucyl, or D-alanyl and wherein Z is glycyl-NHR_1 or NHR_1 wherein R_1 is H, lower alkyl or haloloweralkyl.

72) Th combination according to any one of claims 62 to 71 wherein aminoglutethimide and/or ketokonazole are used as inhibitors of sex steroid biosynthesis.

73) The combination according to any one of claims 62 to 72 wherein the antiestrogen is (Z)-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-N,N-dimethylethanamine or LY-117018.

FIG. 1

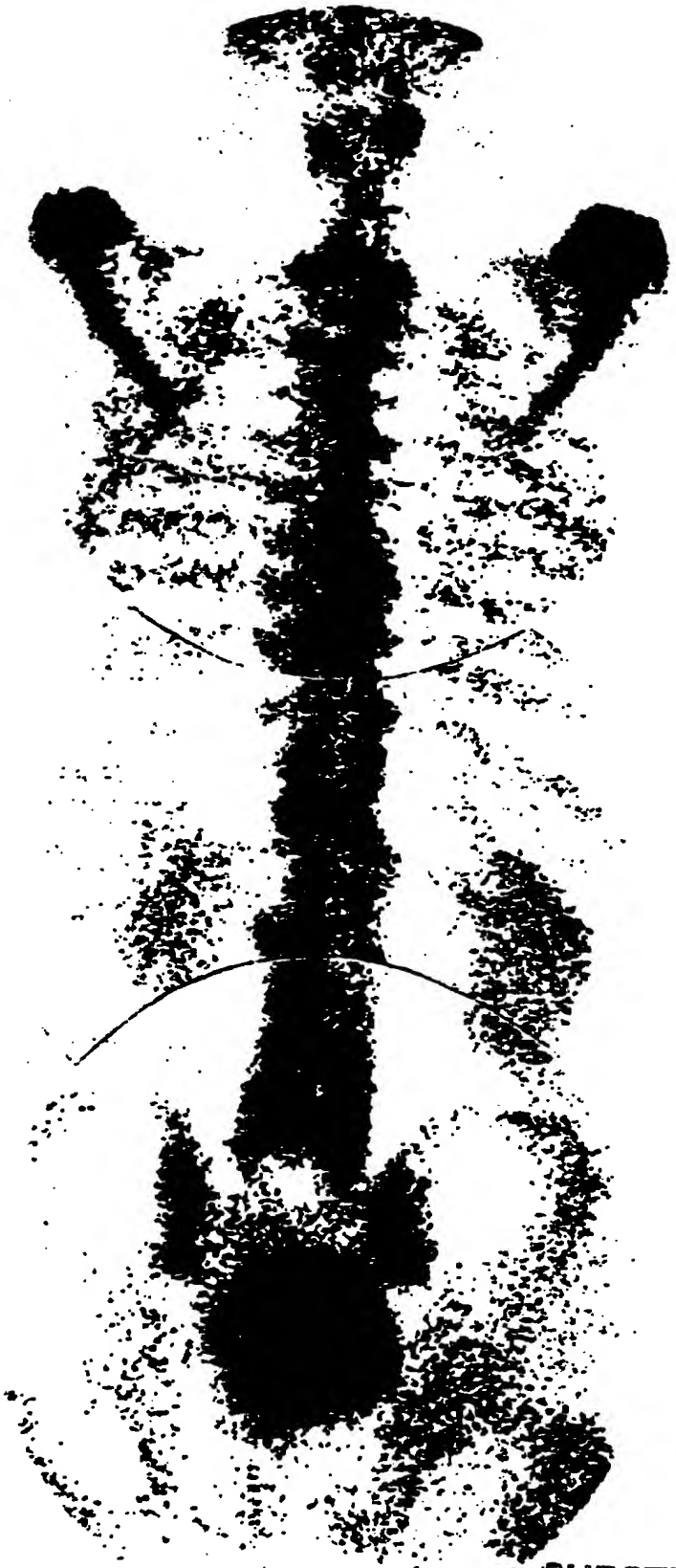


FIG. 2

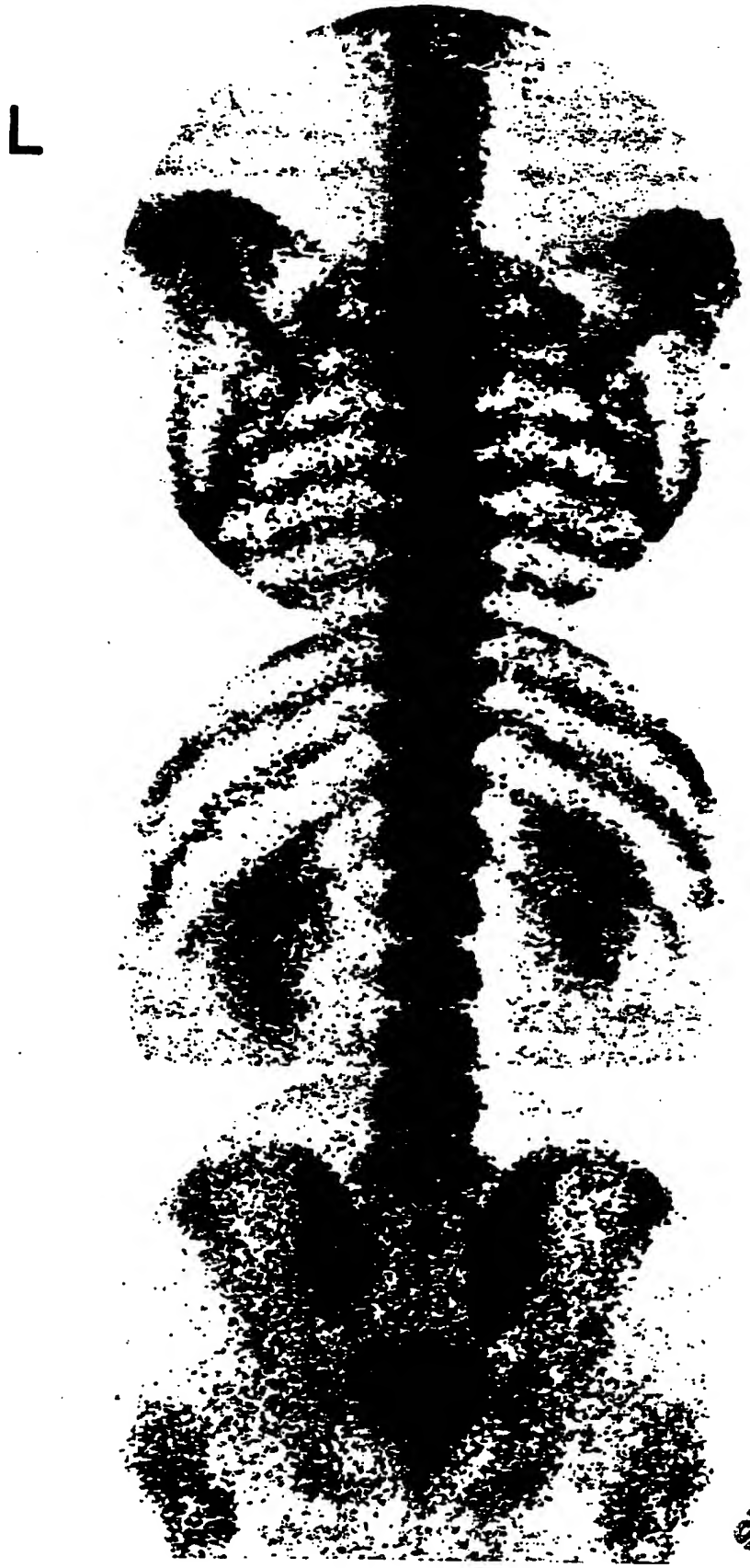


FIG. 3

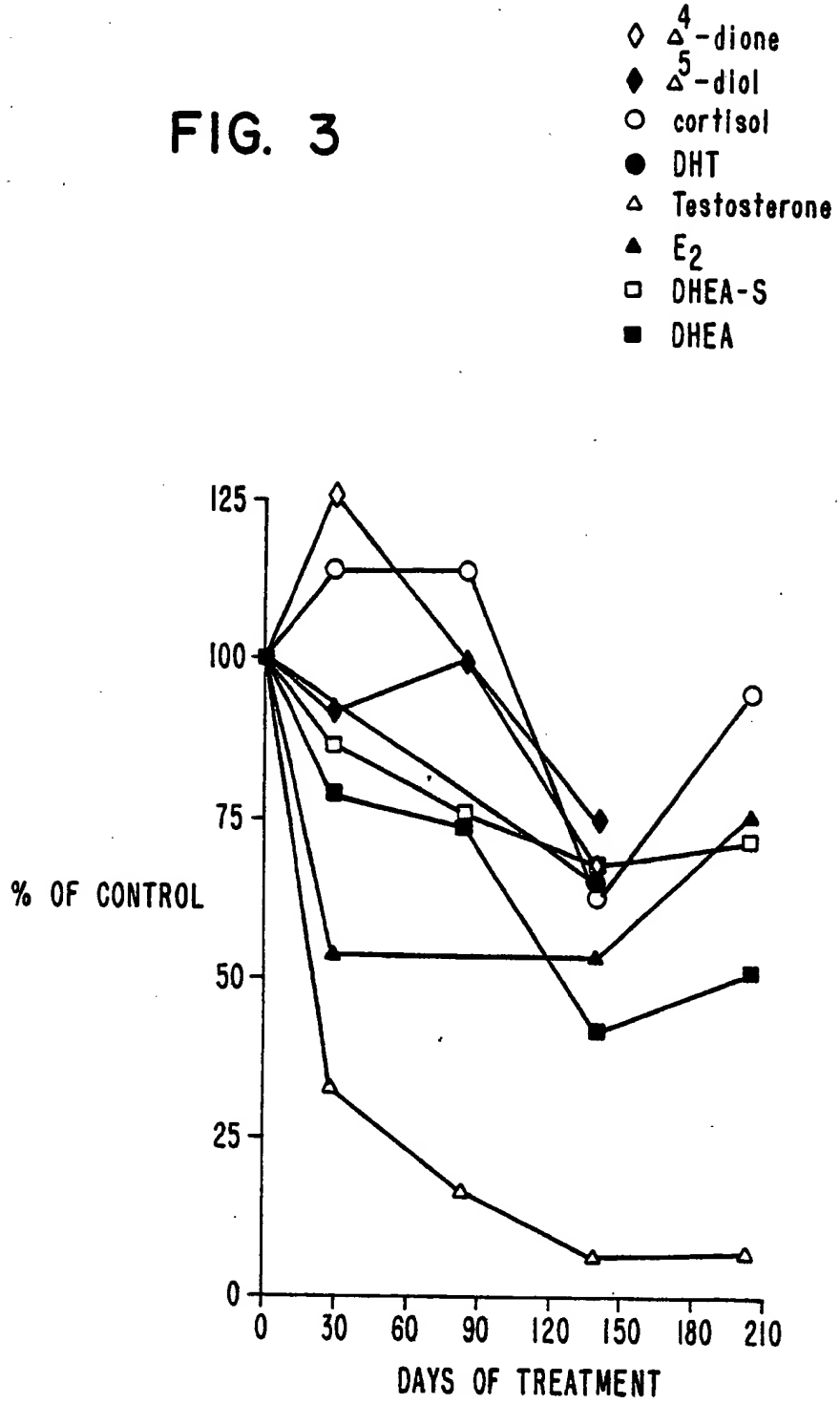
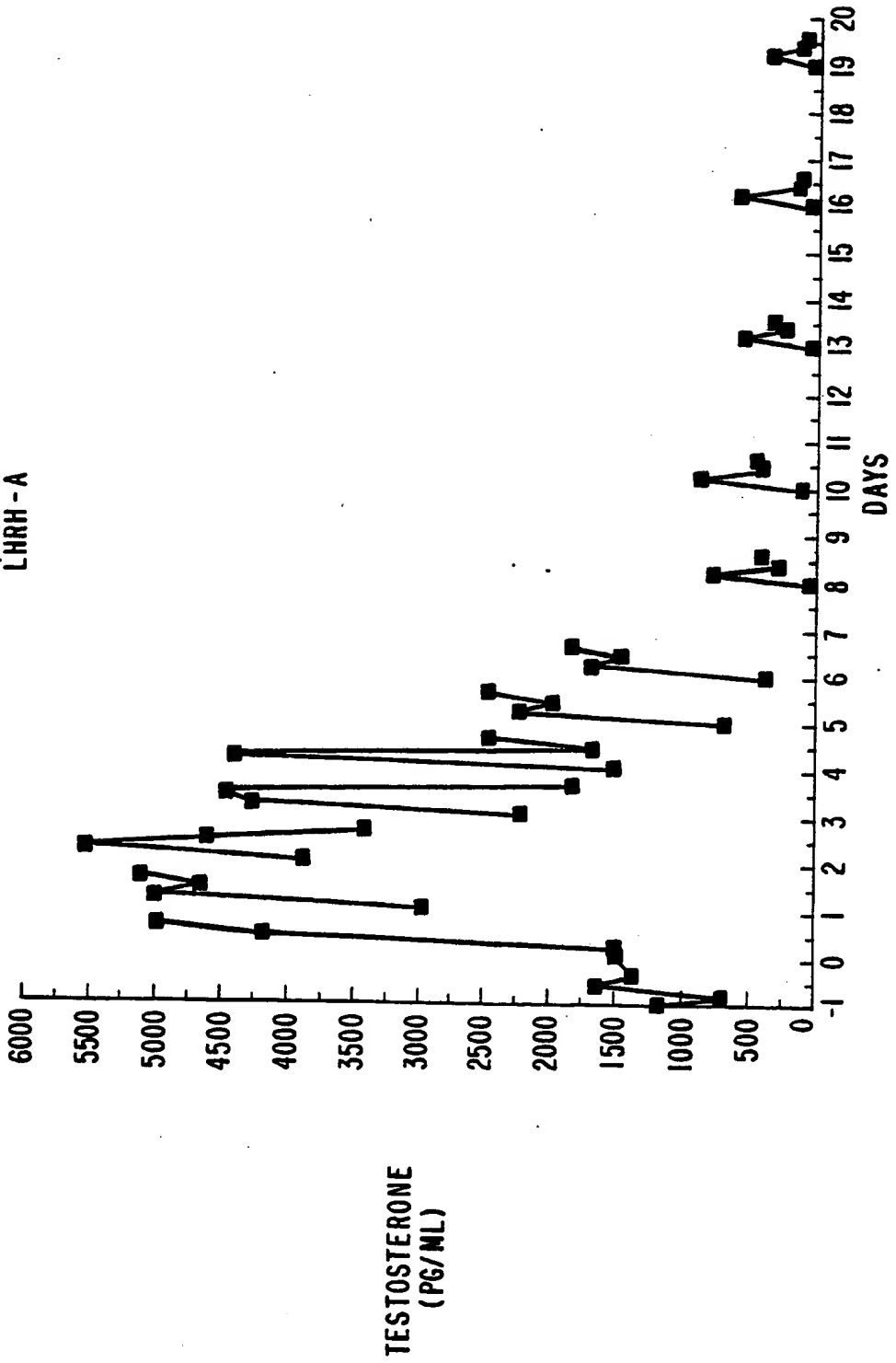

◊ Δ4-dione
♦ Δ5-diol
○ cortisol
● DHT
△ Testosterone
▲ E2
□ DHEA-S
■ DHEA
125
100
75
50
25
0
% OF CONTROL
0
30
60
90
120
150
180
210
DAYS OF TREATMENT

FIG. 4

LHRH-A


TESTOSTERONE (PG/ML)
6000
5500
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4500
4000
3500
3000
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2000
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0
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
DAYS

FIG. 5

LHRH-A + KETOCONAZOLE

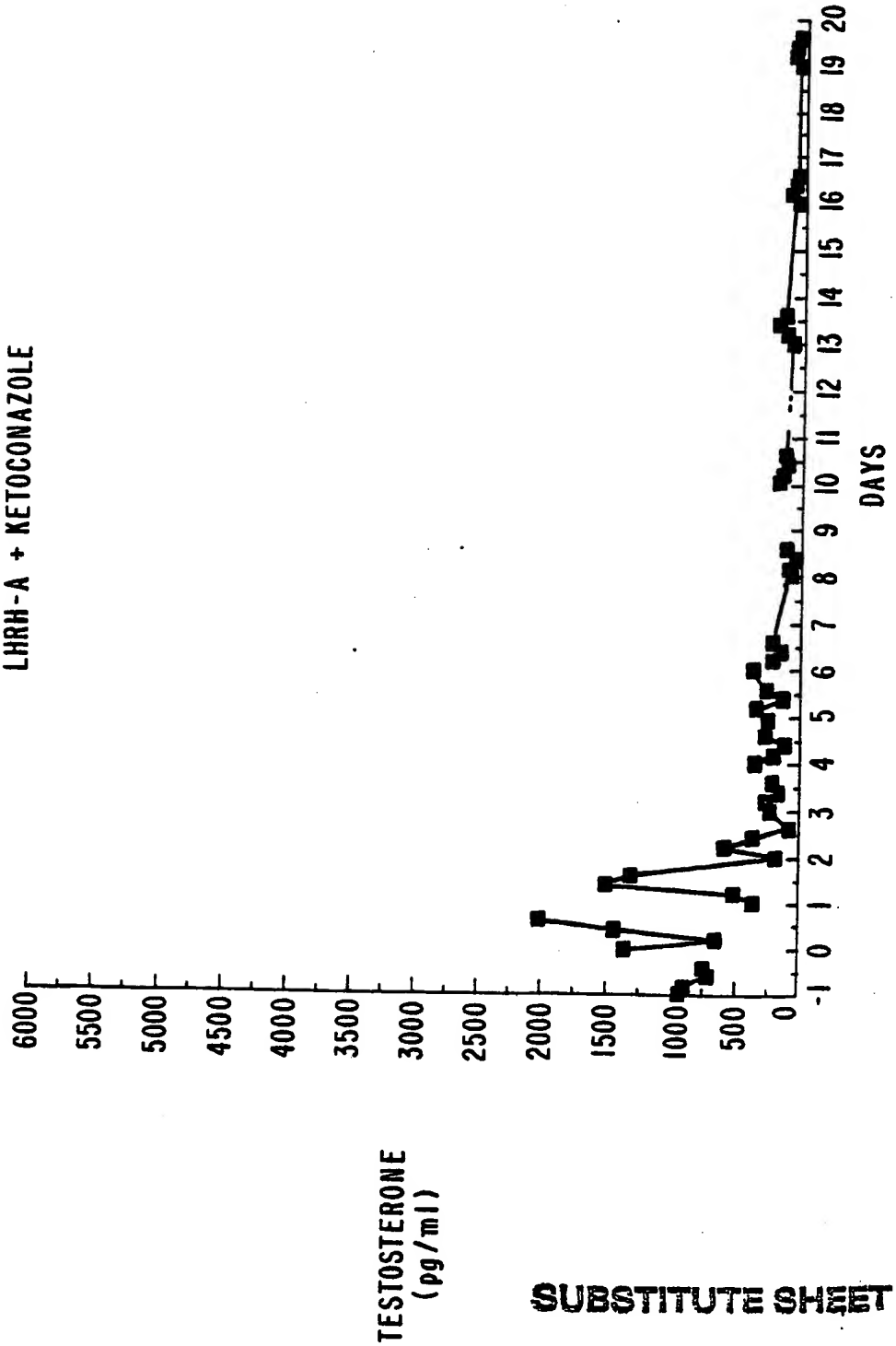
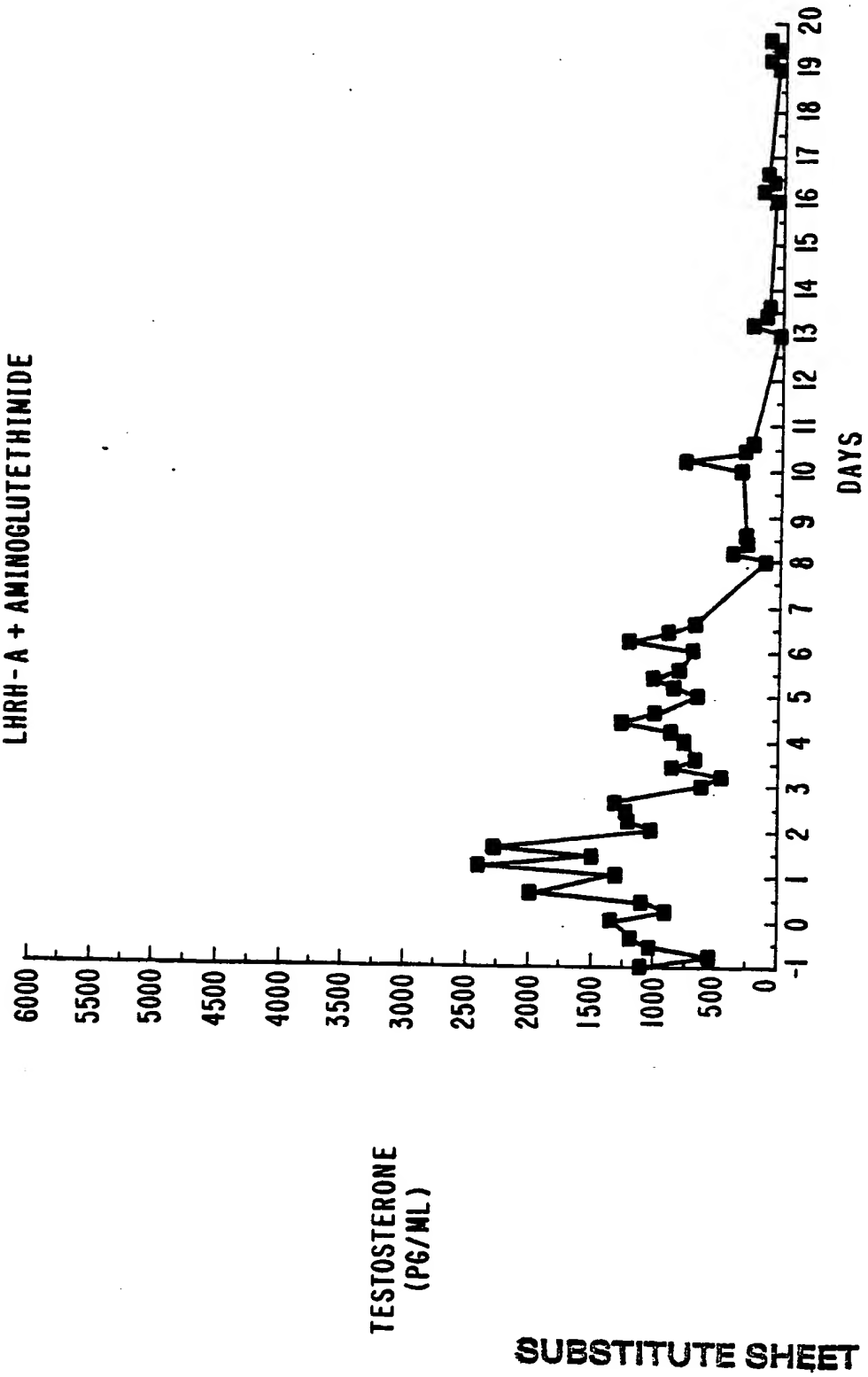


FIG. 6


LHRH-A + AMINOGLUTETHIMIDE
TESTOSTERONE (PG/ML)
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
DAYS

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 85/01454

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: A 61 K 31/445; A 61 K 31/495; A 61 K 31/165; A 61 K 31/415; //(A 61 K 31/445, 31/415, 31/165) (A 61 K 31/495, 31/415, 31/165)

II. FIELDS SEARCHED

Minimum Documentation Searched 7	
Classification System	Classification Symbols
IPC[4]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched 8

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
A	US, A, 4329364 (RUDOLPH O. NERI) 11 May 1982, see column 6, lines 35-40; claim 1 (cited in the application)	62-73
A	US, A, 4097578 (JACQUES PERRONNET) 27 June 1978, see column 4, lines 36-67; claims 1-7 (cited in the application)	62-73

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
7th November 1985	04 DEC. 1985
International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	G.L.M. Kruydenberg

Form PCT/ISA/210 (second sheet) (January 1985)

FURTHER INF RMATION CONTINUED FR M THE SE ND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 1-14, 39-61, because they relate to subject matter not required to be searched by this Authority, namely:

Claims 1-14 and 39-61.

See Rule PCT 39.1.(iv).

Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/US 85/01454 (SA 10389)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 25/11/85

The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A- 4329364	11/05/82	US-A-	3995060	30/11/76
US-A- 4097578	27/06/78	NL-A-	7611576	03/05/77
		FR-A,B	2329276	27/05/77
		BE-A-	847742	28/04/77
		DE-A-	2649925	12/05/77
		LU-A-	76085	31/05/77
		CH-A-	599164	12/05/78
		GB-A-	1518444	19/07/78
		AU-A-	1909576	04/05/78
		JP-A-	52057176	11/05/77
		AU-B-	500542	24/05/79
		CA-A-	1086751	30/09/80
		SE-A-	7610859	30/04/77
		SE-B-	430502	21/11/83